



TITLE:

Oxidations of some nitrogen and sulfur-containing heterocycles with biochemical significance( Dissertation\_全文 )

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CITATION:

Saito, Isao. Oxidations of some nitrogen and sulfur-containing heterocycles with biochemical significance. 京都大学, 1968, 工学博士

ISSUE DATE:

1968-11-25

URL:

<https://doi.org/10.14989/doctor.k853>

RIGHT:

OXIDATIONS OF  
SOME NITROGEN-AND SULFUR-CONTAINING  
HETEROCYCLES  
WITH BIOCHEMICAL SIGNIFICANCE

*1968*

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*KYOTO UNIVERSITY*

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## Preface

In the present thesis, the author's studies are collected, which have been carried out under the guidances of Professor Teruo Matsuura and Professor Saburo Fukui at Kyoto University during 1963 - 1968. The studies contain the investigation on the oxidation of some nitrogen-and sulfur-containing heterocyclic compounds, particularly therein photosensitized oxygenation, in relation to the biological oxidation.

The author is indebted to Professor Teruo Matsuura for his invaluable assistance in directing and encouraging this work.

Grateful acknowledgement is also made to Professor Saburo Fukui for his continuing guidance and encouragement during the course of studies.

The author wishes to express heartfelt thanks to Dr. Akira Nishinaga for his valuable advices and discussions in the whole course of the studies.

Finally, the author's grateful thanks are also due to the members of research group of Professor Teruo Matsuura.

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August, 1968.

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## GENERAL INTRODUCTION

Recently a new research area has emerged from the application of the organic chemical approach to the problems in chemical transformation found in biochemistry. Organic chemical investigation on the oxidation of natural components may often give an useful information for the elucidation of the detailed chemical mechanisms of biological oxidations of them. For this purpose autoxidation and oxidation with one electron-transfer oxidizing agent such as ferricyanide are commonly used<sup>1</sup>. Photosensitized oxygenation, which seems to be closely related to some biological oxidation catalyzed certain enzymes such as oxygenases and some oxidases<sup>2</sup>, is also important method for this purpose.

The photosensitized oxygenation of organic compounds has been studied extensively by many workers and represents a very smooth method for introducing oxygen in a specific fashion into organic compounds. For the photosensitized oxygenna-

tion two mechanisms have been proposed. One involves a excited sensitizer-oxygen complex<sup>3</sup> and the other, excited singlet molecular oxygen.<sup>4</sup> The reaction can be distinguished from the familiar radical oxidation. Recently photosensitized oxygenation has drawn much attention in the area of organic photochemistry in view of the electrophillic character of singlet oxygen and the mode of peroxide formation.<sup>4</sup>

Photosensitized oxygenation is also important in the field of biochemistry, especially in photobiology. The irradiation of certain biological systems, such as bacteria, virus, proteins and nucleic acids, in the presence of a sensitizing dye and oxygen induces various biological effects, such as mutation, denaturation and carcinogenic effects. These phenomena are well known as photodynamic actions.<sup>5</sup> Thus, transforming deoxyribonucleic acid (DNA) is deactivated by visible light in the presence of a sensitizer and oxygen.<sup>6</sup> This is known to be mainly due to the selective destruction of guanine residues in DNA by photosensitized oxygenation.<sup>6</sup>

In order to contribute to the elucidation of the mechanisms of these photodynamic inactivation of DNA, a systematic investigation on the photosensitized

oxygenation of various purines including guanine has been carried out and presented in Chapter I. From the results obtained it is concluded that the peroxide intermediates, which are initially formed by the attack of singlet oxygen to the purines, give various degradation products and that the nature of the peroxide is depending upon the structural feature of the imidazole moiety of the purines.

In Chapter II the photosensitized oxygenation and alkaline ferricyanide oxidation of quinoxaline-2, 3-diols, which are known to be catabolic intermediates of riboflavin, has been examined as a possible model for the biological oxidative decomposition of riboflavin. Mechanisms of these oxidation reactions are discussed in connection with the enzymatic degradation of riboflavin.

In Chapter III oxidation including photosensitized oxygenation of lipoic acid has been carried out in relation to the elucidation of the structure of the metabolites of lipoic acid. The microbiological and pyruvate oxidation activities of the oxidation products are also presented. Furthermore, the models

for enzymatic acyl-transfer reactions, in which lipoic acid plays an important role, are also demonstrated.

Finally, in Chapter IV photosensitized oxygenation of thiazoles is mentioned. Possible mechanisms involving peroxide intermediate are discussed in this chapter.

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6. See ref. 4-7 of Chapter I, Introduction.

CHAPTER I  
PHOTOSENSITIZED OXYGENATION  
OF PURINE DERIVATIVES

1. Introduction

The numerous investigations have demonstrated that any living systems such as organism, virus, enzymes, proteins and nucleic acids are susceptible to photosensitized oxidation in the presence of light, oxygen and a sensitizing dye. To these phenomena the term photodynamic action has been applied.<sup>1</sup> The fact that substances of biological interest such as chlorophyll, porphyrins, flavins etc. are photodynamic, is suggesting that photodynamic action might play an important role in physiological process.<sup>2</sup> Furthermore, much attention, at present, is drawn to photodynamic action in relation to some diseases brought about by drugs and to carcinogenic process initiated by photodynamic chemicals.<sup>2,3</sup>

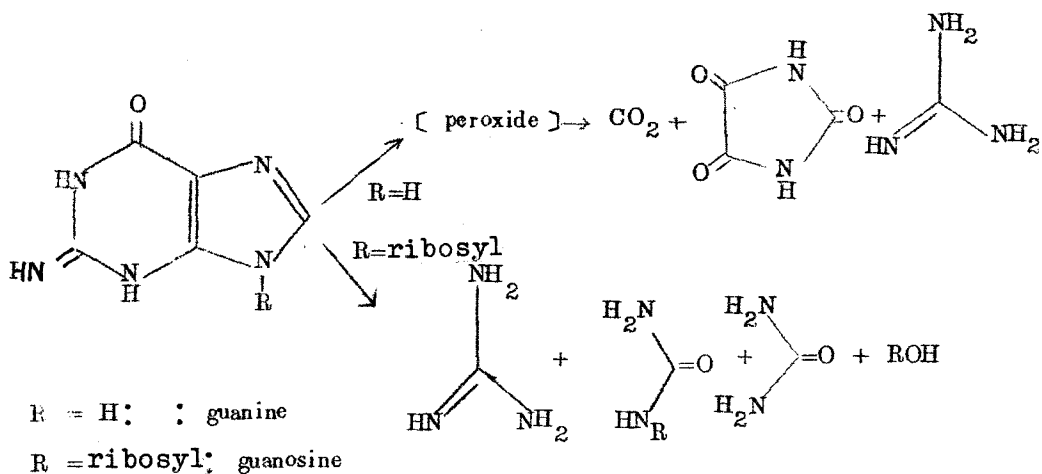
In connection with these photodynamic inactivations of organisms and virus, a great deal of evidences have accumulated which suggest that photosensitized

oxygenation of nucleic acids is involved.<sup>1</sup> It seems necessary, therefore, in order to pursue the nature of these photodynamic actions, to understand some of the chemical mechanisms involved in the photo-sensitized oxygenation of nucleic acids.

The photodynamic inactivation of nucleic acids has been found to be mainly due to the selective degradation of guanine residues in deoxyribonucleic acids (DNA).<sup>4,5,6</sup> Thus, by using DNA containing 8-<sup>14</sup>C -guanine and 8-<sup>14</sup>C -adenine it has been demonstrated that at least 80% of the guanine was selectively destroyed by lumichrome-sensitized oxidation and that neither adenine nor pyrimidine bases was destroyed under the conditions.<sup>7</sup>

Sussenbach and Behrends<sup>8</sup> reported that lumichrome-sensitized photooxidation of guanine in an aqueous solution results in a mixture of products from which carbon dioxide, parabanic acid, and guanidine can be detected. They proposed the intermediary formation of a peroxide for which chemical structure remains unknown. On the other hand, Sastry and Gordon<sup>9</sup> reported that methylene blue-sensitized photooxidation

of guanosine afforded guanidine, ribosylurea, urea, and ribose.



Since the results indicate that guanine and guanosine are extensively degraded by photosensitized oxygenation, it seems difficult to clarify the nature of these reactions. Therefore, a systematic investigation on the photosensitized oxygenation of purine derivatives has been carried out, in order to contribute to the elucidation of the chemical mechanisms of photodynamic inactivation of guanine residues in DNA.



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## 2. Photosensitized Oxygenation of N-unsubstituted Hydroxypurines

### 2.1. Introduction

In connection with the photodynamic inactivation of deoxyribonucleic acid,<sup>1</sup> the photosensitized oxygenation of guanine and related purine derivatives has recently drawn much attention. Irradiation of guanine and guanosine by visible light in the presence of sensitizing dyes and oxygen results in destruction of the molecule to give various products,<sup>2</sup> and details of the reaction pathway remain unknown. Other hydroxylated purine derivatives, such as uric acid, xanthine, and hypoxanthine, are also known to consume oxygen under similar conditions.<sup>3,4,5</sup> Recently Anmann and Vynch<sup>6</sup> have reported that uric acid, when submitted to the methylene blue-sensitized photooxygenation in aqueous ethanol, yields cyanuric acid, allantoin, parabanic acid, urea, and other three unidentified compounds, although all these substances were identified only on paperchromatograms. Without a sensitizer, triuret was

obtained in the photooxidation of uric acid under the influence of ultraviolet light.<sup>7</sup> In order to elucidate the detailed chemical mechanisms of the photosensitized oxygenation of those hydroxylated purines, the photosensitized oxygenation of three N-unsubstituted hydroxypurines, i.e., xanthine (1), uric acid(4), and 8-methylxanthine(7), in alkaline media using rose bengal as a sensitizer, has been carried out.

## 2.2. Results and Discussion

When an aqueous alkaline solution of xanthine(1) was irradiated in the presence of rose bengal under bubbling oxygen, 1.05 mole equivalent of oxygen was consumed. Acidification of the reaction mixture liberated carbon dioxide (1.0 mole equivalent), which was determined by converting into barium carbonate. From the acidified mixture triuret (2) (5%) and allantoin (3) (41%) were obtained as major products. Triuret was identified by its synthesis<sup>8</sup> and by its derivation into cyanuric acid.

Uric acid (4) also consumed 1.1 mole equivalent

of oxygen under similar conditions. In this case, the yields of products were dependent upon pH during isolation of the products. Isolation of the photoproducts at pH 2 yielded triuret (2), sodium oxonate (5) allantoxaidin (6), and carbon dioxide, in 20, 30, 15, and 85% yields, respectively. When the isolation was made at pH 5.0, the yield of sodium oxonate (5) was increased up to 40% at the expense of carbon dioxide (25%) and no allantoxaidin (6) was obtained. On the other hand, photooxygenation of uric acid in the presence of a large excess of alkali followed by the isolation of the products at pH 5.0, afforded triuret (3, 8%), sodium oxonate (5, 69%), and carbon dioxide (10%). Since it is known that sodium oxonate (5) is converted to allantoxaidin (6) by treatment with a strong acid,<sup>9,10</sup> it is concluded that allantoxaidin formed from uric acid is the secondary product which was formed in the course of isolation.

Photosensitized oxygenation of 8-methylxanthine (7) in the presence of 1.1 mole equivalent of alkali resulted in consumption of 0.95 mole equivalent of oxygen to give a complex mixture, which was found, by paper

chromatography, to consist of at least six compounds. From the mixture, acetamide (6%), sodium oxonate (5, 25%), and carbon dioxide (53%) were obtained. All the above results are summarized in Table 1.

In the absence of the sensitizer, none of the substrates consumed oxygen upon irradiation and the starting materials were recovered quantitatively, indicating that the presence of a sensitizer is a requisite condition for the present photooxygenation. Although Weber has shown that some purine derivatives form a complex with riboflavin in solution,<sup>11</sup> no spectroscopic evidence was obtained for the formation of a charge-transfer complex between rose bengal and any of the substrate. The above results demonstrate that the excited state, possibly a triplet state,<sup>12</sup> of the sensitizer may be involved in the present reaction. Recently the suggestion that singlet excited oxygen is involved in dye-sensitized photooxygenation of certain organic compounds was strengthened by several workers.<sup>13</sup> Although mechanisms involving an energy transfer<sup>14</sup> and an electron transfer<sup>15</sup> between triplet excited sensitizer and purine derivatives

Table 1. Photosensitized oxygenation of hydroxypurines.

Substrate	NaOH added (mole)	O <sub>2</sub> consumed (mole)	CO <sub>2</sub> liberated (mole)	pH change	Products (% yield)
Xanthine ( <u>1</u> )	0.5 <sup>a</sup>	1.0	1.0	11.7 - 8.8	<u>2</u> (5) <u>3</u> (41)
Uric acid ( <u>4</u> )	1.6 <sup>a</sup>	1.1	0.85	13.0 -12.7	<u>2</u> (20) <u>5</u> (30) <u>6</u> (15)
	1.6 <sup>b</sup>	1.0	0.25	13.0 -12.6	<u>2</u> (29) <u>5</u> (40)
	13.5 <sup>b</sup>	1.0	0.1	13.2 -13.1	<u>2</u> ( 8) <u>5</u> (69)
8-Methyl- xanthine ( <u>7</u> )	1.1 <sup>a</sup>	0.95	0.53	12.8 -12.4	<u>5</u> (25) <u>8</u> ( 6)

<sup>a</sup> The reaction mixture was adjusted to pH 2.0 with 2N HCl before the isolation of the products.

<sup>b</sup> The reaction mixture was adjusted to pH 5.0 with acetic acid before the isolation of the products.

have been postulated, recent results obtained in the oxygenation of N-alkylated purine derivatives with singlet oxygen<sup>16</sup> strongly suggest that, in the present photosensitized oxygenation, singlet oxygen may attack the ground state molecule of the substrates.\* Thus, it appears that the attack of singlet oxygen may result in the initial formation of a peroxide intermediate, either a cyclic peroxide or a hydroperoxide, depending upon the nature of the starting material. Possible sequences for the photosensitized oxygenation of the hydroxypurines, 1, 4, and 7, are illustrated in Scheme 1, 2, and 3.

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\* Oxidation with singlet oxygen generated from hydrogen peroxide and hypochlorite<sup>13</sup> would not be applied to these hydroxypurines which are soluble in the solvent system employed only in the presence of alkali. Because these hydroxypurines are known to be easily oxidized with hydrogen peroxide alone under alkaline conditions to give various products.<sup>9,10</sup>

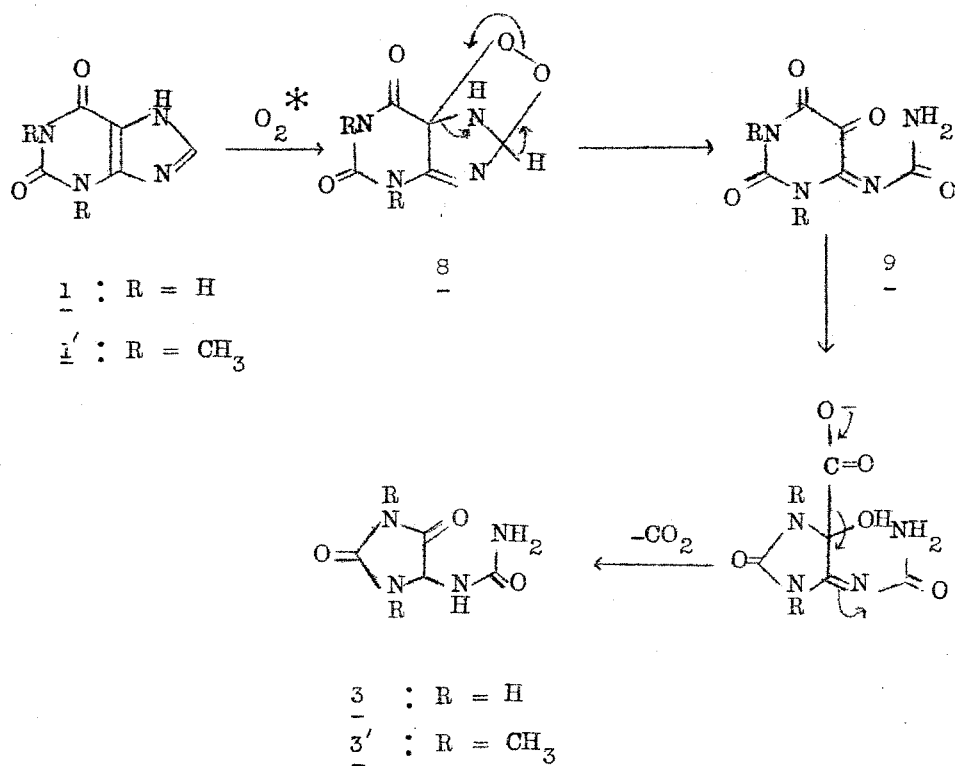
The formation of allantoin (2), the major product from xanthine (1), is well rationalized by a pathway involving a cyclic peroxide 8 (Scheme 1). Such a cyclic peroxide is known to be an initial product in the photosensitized oxygenation of cyclic dienes,<sup>17</sup> and it has been postulated as an intermediate in the photosensitized oxygenation of certain types of five-membered N-heteroaromatics, i. e., pyrroles<sup>18</sup> and oxazoles.<sup>19,20</sup> Peroxide 8 then rearranges to form an alloxanimide derivative 9 which is, on benzylic acid-type rearrangement\* and subsequent decarboxylation, converted to allantoin (2). The formation of 1,3-dimethylallantoin (3') in the photosensitized oxygenation of theophylline (1')<sup>4</sup> is quite analogous to our results obtained with xanthine (1).

In the photosensitized oxygenation of uric acid (4), the attack of singlet oxygen would form a hydroperoxide 10 or 11 (Scheme 2). This type of hydroperoxide is generally accepted as an intermediate in

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\* An aqueous alkaline solution of alloxan decomposes to give alloxanic acid, 5-hydroxyhydantoin-5-carboxylic acid.<sup>21</sup>





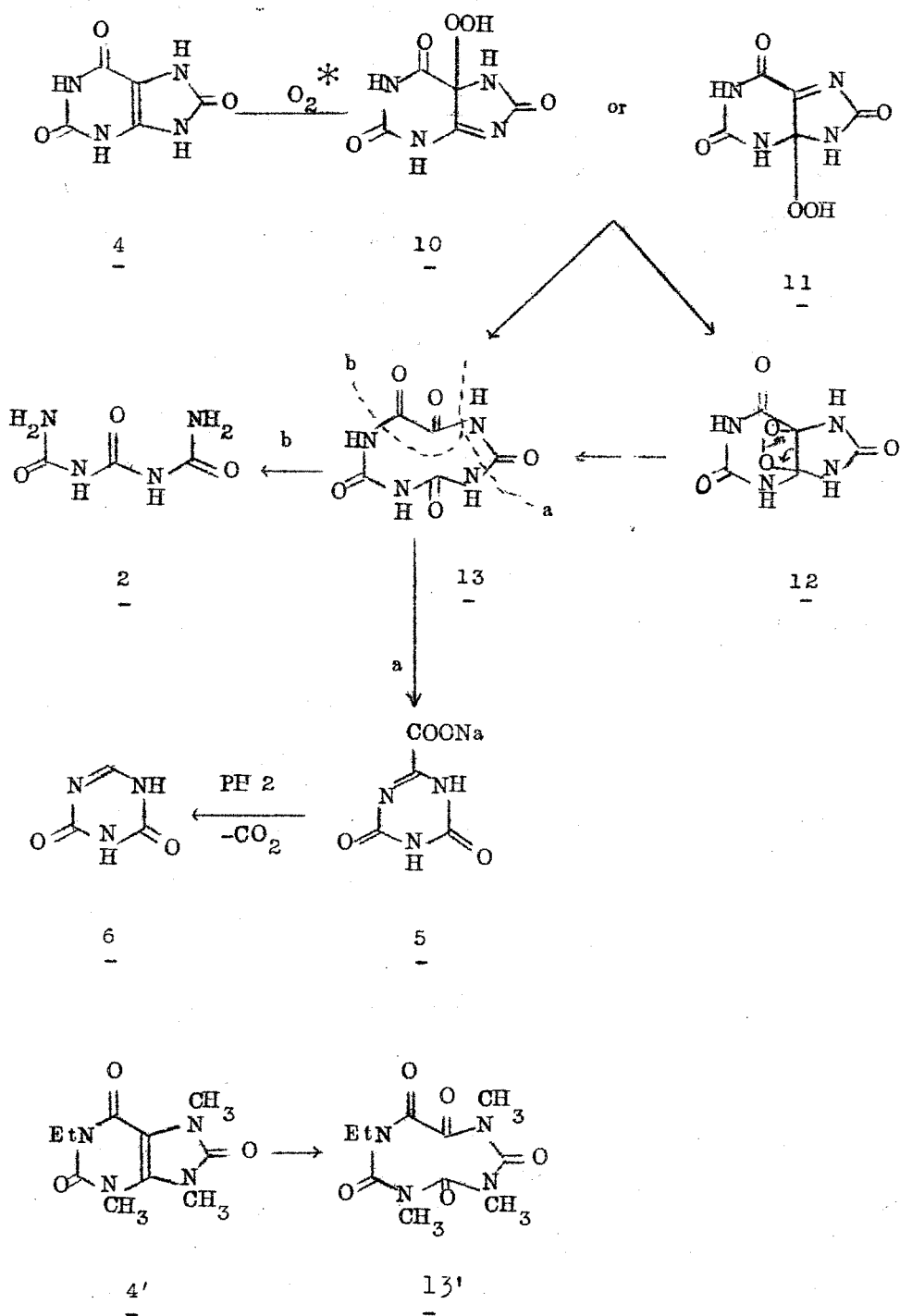
Scheme 1

the photooxygenation of certain N-heterocycles having a moiety  $-C=C-NH-$ , i. e., imidazoles,<sup>22,23</sup> pyrroles,<sup>24,25</sup> and indoles.<sup>26</sup> The 5,6-bond of hydroperoxide 10 or 11 is cleaved, concertedly or via a four-membered cyclic peroxide 12, to form a nine-membered intermediate 13, which could be hydrolyzed to give sodium

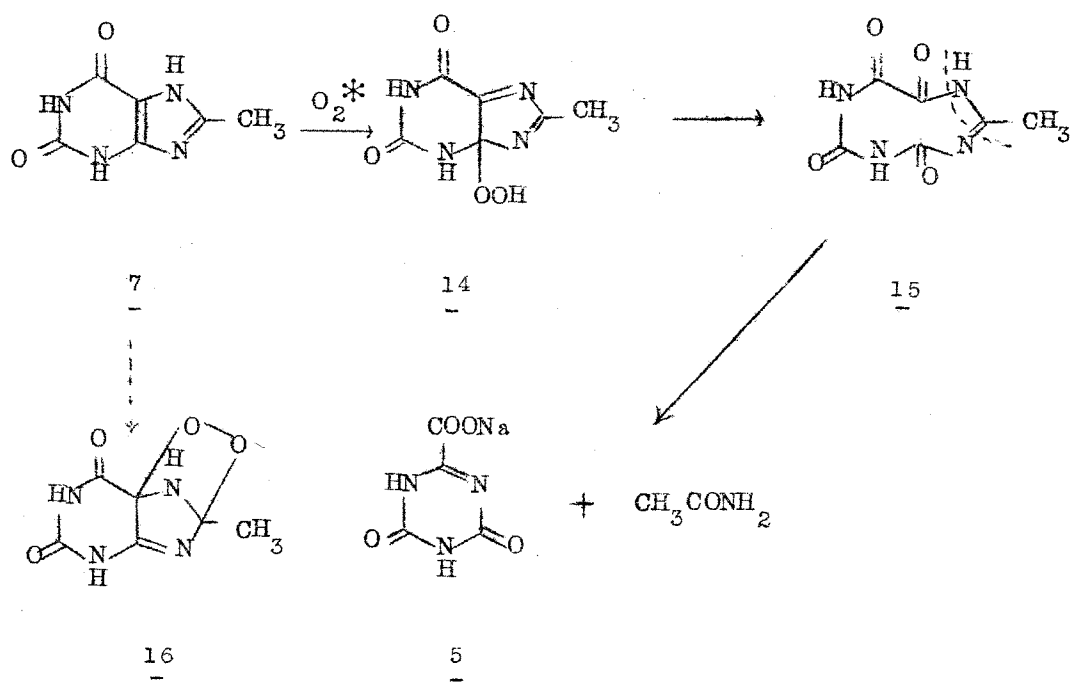
oxonate (5) via path a and triuret (2) via path b.

Nine-membered compounds analogous to 13 is known to be obtained as one of the products in the photosensitized oxygenation of fully N-alkylated uric acids, e. g., 13' from 1-ethyl-3,7,9-trimethyluric acid (4').<sup>16</sup> As already discribed, sodium oxonate (5) when treated with hydrochloric acid is easily decarboxylated to form allantoxaidin (4).

Finally, a hydroperoxide intermediate 14 may also account for the photooxygenation of 8-methylxanthine (7)(Scheme 3). Hydroperoxide 14 would give analogously a cyclic intermediate 15 which is then hydrolyzed to acetamide and sodium oxonate (5). However, a possibility for the formation of cyclic peroxide 16 analogous to 8 cannot be eliminated. Peroxide 16 will lead to the intermediate 15 and also to other unidentified products.



Scheme 2



Scheme 3

## 2.3. Experimental

### 2.3.1. General Procedure for Photosensitized Oxygenation.

A solution of the substrate and rose bengal in NaOH aq was irradiated using a 100 W high-pressure mercury lamp with a Pyrex cooling jacket at room temp. During irradiation oxygen was bubbled through the solution in a closed circulating system and the consumption of oxygen was determined manometrically. After the reaction mixture had been acidified with 2N HCl to appropriate pH, nitrogen was bubbled through the mixture and carbon dioxide liberated was trapped with  $\text{Ba}(\text{OH})_2$  aq. The yield of carbon dioxide was determined by weighing  $\text{BaCO}_3$  precipitated. The products were isolated as described below. The results are summarized in Table 1.

### 2.3.2. Photooxygenation of Xanthine (1).

A solution of xanthine (1.0 g, 6.6 mmoles) and rose bengal (20 mg) in 0.022N NaOH (150 ml, 3.3 mmoles) was photooxidized. After the absorption of oxygen (155 ml) had ceased (1 hr), the mixture was acidified

to pH 2.0. Carbon dioxide (6.6 mmoles) was liberated. The acidified mixture was treated with Norit to remove rose bengal, then was concentrated under red press to 30 ml depositing triuret (2) as a yellow solid (50 mg, 5%) which was crystallized from water, m.p. 230-238<sup>o</sup> (lit.<sup>8</sup> 231-235<sup>o</sup>). The IR spectrum was identical to that of an authentic sample.<sup>8</sup> The identity of the compound was further confirmed by its derivation to cyanuric acid.<sup>7,8</sup>

The mother liquor was evaporated to dryness to give a residue which was crystallized from water to give allantoin (3) (0.42 g, 41%) as crystals, m.p. 235-236<sup>o</sup>. The IR spectrum was identical to that of an authentic sample.

### 2.3.3. Photooxygenation of Uric Acid (4)

(a) Isolation of Products at pH 2.0. A suspension of uric acid (2.0 g, 12 mmoles) in 0.16N NaOH (120 ml, 19.2 mmoles) containing rose bengal (20 mg) was photo-oxidized (1.5 hr). The mixture became a clear solution as oxygen was consumed. A white solid deposited during irradiation was identified as triuret (50 mg). After

oxygen (300 ml) had been consumed, the mixture was acidified to pH 2.0, liberating carbon dioxide (10.2 mmoles). Removal of the solvent left a solid mass which was crystallized from water (35 ml) to give triuret (0.28 g; total 0.33 g, 20%). Concentration of the mother liquor to 10 ml afforded crystals (0.63 g, 30%), m.p.  $> 300^{\circ}$ , which were identified as sodium oxonate<sup>9,27</sup> (by IR).  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  230 m $\mu$  ( $\epsilon$  4750),  $\lambda_{\text{max}}^{0.01\text{N NaOH}}$  252 m $\mu$  ( $\epsilon$  6780). Further concentration of the mother liquor gave allantoxaidin (0.21 g, 15%), m.p.  $280-282^{\circ}$ , which was identical with a sample prepared by treatment of sodium oxonate with 1N  $\text{H}_2\text{SO}_4$ <sup>9,10</sup> (by IR).  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  232 m $\mu$  ( $\epsilon$  5600),  $\lambda_{\text{max}}^{0.01\text{N NaOH}}$  250 m $\mu$  ( $\epsilon$  7200),  $\nu_{\text{max}}^{\text{Nujol}}$  1800 and 1730  $\text{cm}^{-1}$ .

(b) Isolation of Products at pH 5.0. The mixture, which was obtained by the photooxygenation of uric acid as described above, was acidified to pH 5.0 with acetic acid. Triuret (0.50 g, 29%), sodium oxonate (0.70 g, 40%), and carbon dioxide (3 mmoles) were obtained by the similar procedure. No allantoxaidin was detected.

(c) Photooxygenation in the Presence of a Large

Excess of NaOH. A solution of uric acid (5.0 g, 30 mmoles) and rose bengal (20 mg) in 1.6N NaOH (250 ml, 0.405 mole) was photooxidized. From the mixture, which was acidified with acetic acid to pH 5.0, triuret (0.34 g, 8%), sodium oxonate (3.5 g, 69%), and carbon dioxide (3 mmoles) were obtained by the similar procedure.

#### 2.3.4. Photooxygenation of 8-Methylxanthine (7).

A solution of 8-methylxanthine<sup>28</sup> (2.0 g, 12 mmoles) and rose bengal (20 mg) in 0.088N NaOH (150 ml, 13.2 mmoles) was photooxidized. After oxygen (254 ml) had been consumed, the mixture was acidified to pH 2.0 to liberate carbon dioxide (6.35 mmoles). A paper chromatographic analysis using 1-butanol-acetic acid-water (5:1:4) as the solvent showed that products consist of at least six compounds. After removal of the solvent under red press the residue was extracted with boiling benzene (100 ml). Evaporation of the benzene extract left crystals (40 mg, 5.5%) which were identified as acetamide (by IR). The residue insoluble in benzene was dissolved in hot water (50 ml) and the solution was treated with Norit. On cooling the solution



deposited crystals (0.41 g, 22%), which were identified as sodium oxonate (5) (by IR). No other products could be isolated in a pure form.

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### 3. Photosensitized Oxygenation of Hydroxylated 9-Phenylpurines

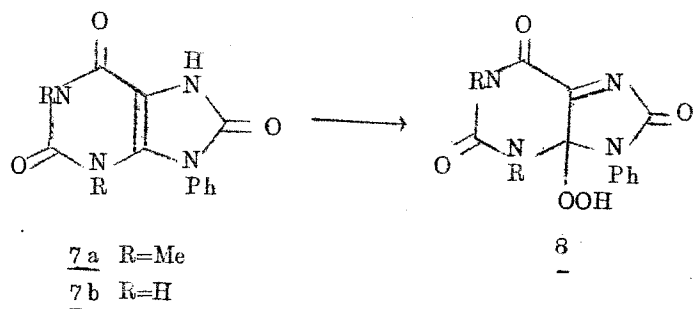
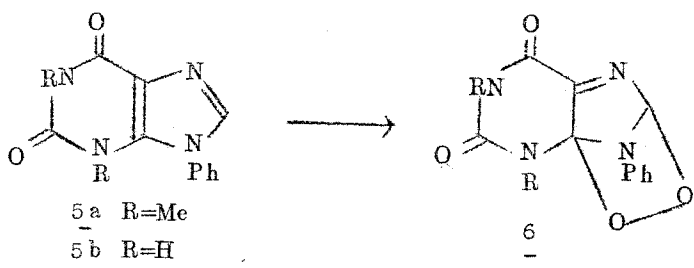
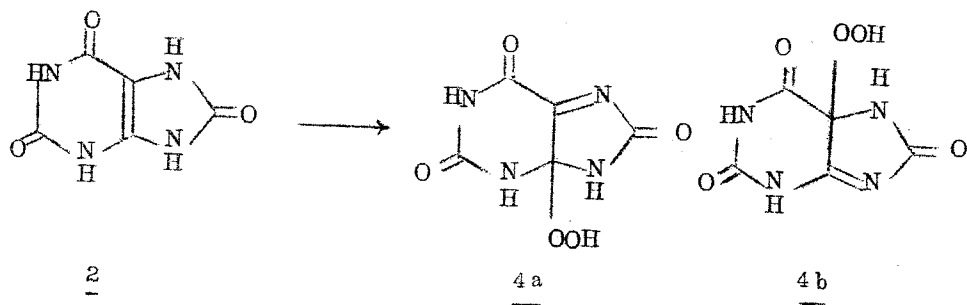
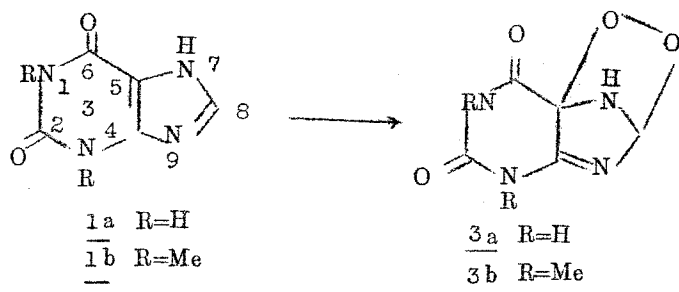
#### 3.1. Introduction

In the previous section,<sup>1,2</sup> it has been shown that the photosensitized oxygenation of hydroxylated purines, including xanthine (1a) and uric acid (2), in an aqueous alkaline solution gives various products which are possibly formed via a peroxide intermediate. In the case, a 5,8-endo-peroxide 3a for the intermediate from 1a and a hydroperoxide 4a or 4b from 2 has been postulated. The formation of 1,3-dimethylallantoin in the photosensitized oxygenation of theophylline (1b)<sup>3</sup> can be also rationalized by considering a similar peroxide intermediate 3b. The peroxides 3 and 4 are analogous to photoperoxides obtained from cyclic conjugated dienes<sup>4</sup> and from olefins bearing an allylic hydrogen,<sup>5</sup> respectively. In contrast to purine derivatives such as 1 in which two double bonds of the imidazole ring are fixed at 4,5- and 8,9-positions, 9-N-substituted purines such as 2 have two double bonds fixed at 4,5- and 7,8-positions. In the latter case it might be expected to result

in the initial formation of a 4,8-endo-peroxide 6 analogous to 3. On the other hand, photosensitized oxygenation of 8-hydroxy-9-N-substituted purines such as 7, in which a double bond is located only at 4,5-position as in 2, would give a 4-hydroperoxy intermediate 8 analogous to 4a.

### 3.2. Results and Discussion

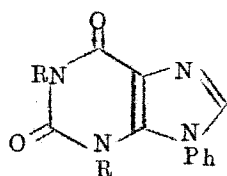
When a methanol solution of 1,3-dimethyl-9-phenylxanthine (5a) was irradiated with a high-pressure mercury lamp (Pyrex) in the presence of rose bengal, 1.3 moles of oxygen was consumed and a crystalline product  $C_{15}H_{18}N_4O_5$  was obtained in 23% yield. Irradiation with visible light in the presence of the sensitizer gave essentially identical results. The NMR spectrum ( $CDCl_3$ ) of the product shows four singlets of two N-methyls and two methoxyls at  $\tau$  6.48, 6.54, 6.80, and 7.45 in addition to a singlet at  $\tau$  3.75 and a multiplet centering at  $\tau$  2.78 corresponding to an NH and a phenyl group. The IR spectrum exhibits intense absorptions at 1720 and  $1675\text{cm}^{-1}$ . Treatment of the product with hydriodic acid gave 1,3-dimethyl-



9-phenyluric acid (7a). Chemical and spectral properties are compatible with structure 9a for the photoproduct. This assignment was confirmed by an independent synthesis of 9a which was obtained by the reaction of 7a with chlorine in methanol applying the known method for the synthesis of 1,3,7,9-tetramethyl-4,5-dimethoxyuric acid from 1,3,7,9-tetramethyluric acid.<sup>6</sup>

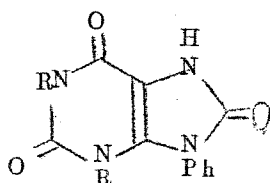
Photosensitized oxygenation of 9-phenylxanthine (5b) in the same manner yielded, after the consumption of 1.1 moles of oxygen, a compound  $C_{13}H_{14}N_4O_5$  in 58% yield. The molecular formula was confirmed by the appearance of its mass parent peak at  $m/e$  306. The NMR spectrum ( $DMSO-d_6$ ) exhibits two methoxy singlets at  $\tau$  6.35 and 6.54, three singlets of three NH protons at  $\tau$  0.75, 3.20, and 3.45, and a multiplet of a phenyl group centering at  $\tau$  2.73. The spectral data suggest structure 9b for the photoproduct. This was confirmed by the methylation of the product with diazomethane yielding 9a.

Photosensitized oxygenation of 9-N-substituted uric acid derivatives (7) also proceeded smoothly.



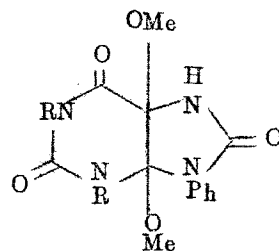
5a R = Me

5b R = H



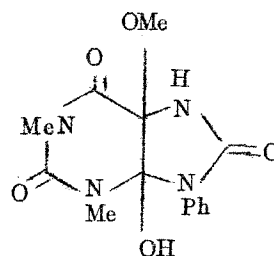
7a R = Me

7b R = H



9a R = Me

9b R = H



10

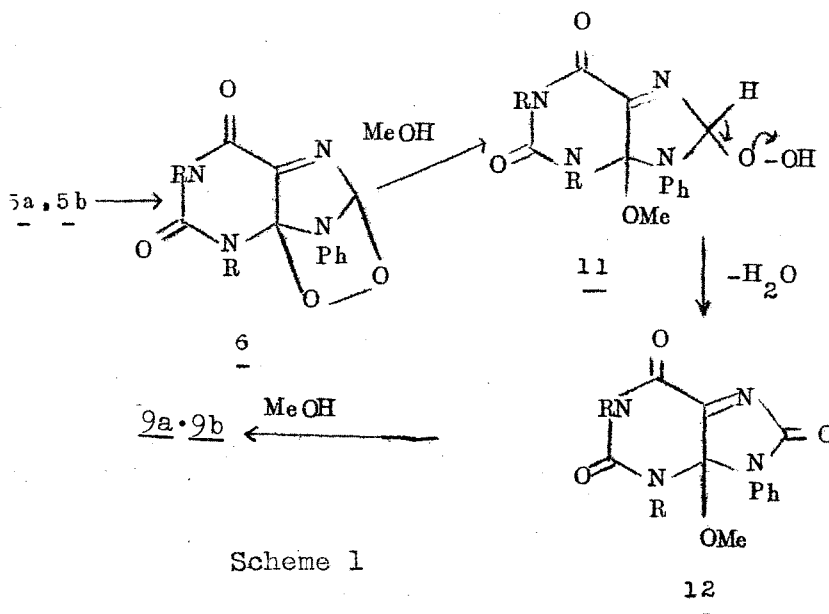
Thus, 9-phenyluric acid (7b) gave, after the consumption of 1.1 moles of oxygen, 4,5-dimethoxy-9-phenyluric acid (9b) in 46% yield. In a similar way, 1,3-dimethyl-9-phenyluric acid (7a) absorbed 1.1 moles of oxygen to



give 9a (2%) and a compound  $C_{14}H_{16}N_4O_5$  (11%). The NMR spectrum ( $CDCl_3$ ) of the latter product shows, in addition to a multiplet of a phenyl group centering at  $\tau 3.0$  and two singlets at  $\tau 2.76$  and  $3.47$  corresponding to OH and NH protons, respectively, a singlet of nine protons at  $\tau 6.66$  suggesting the presence of two N-methyl and an O-methyl groups. Partial hydrolysis of 9a with 2N hydrochloric acid give this trimethylated compound for which, therefore structure 10 was assigned. In the course of the hydrolysis of 9a to 10, a methoxy group at 4-position, which is highly deshielded by a phenyl group at 9-position, might be selectively hydrolyzed.

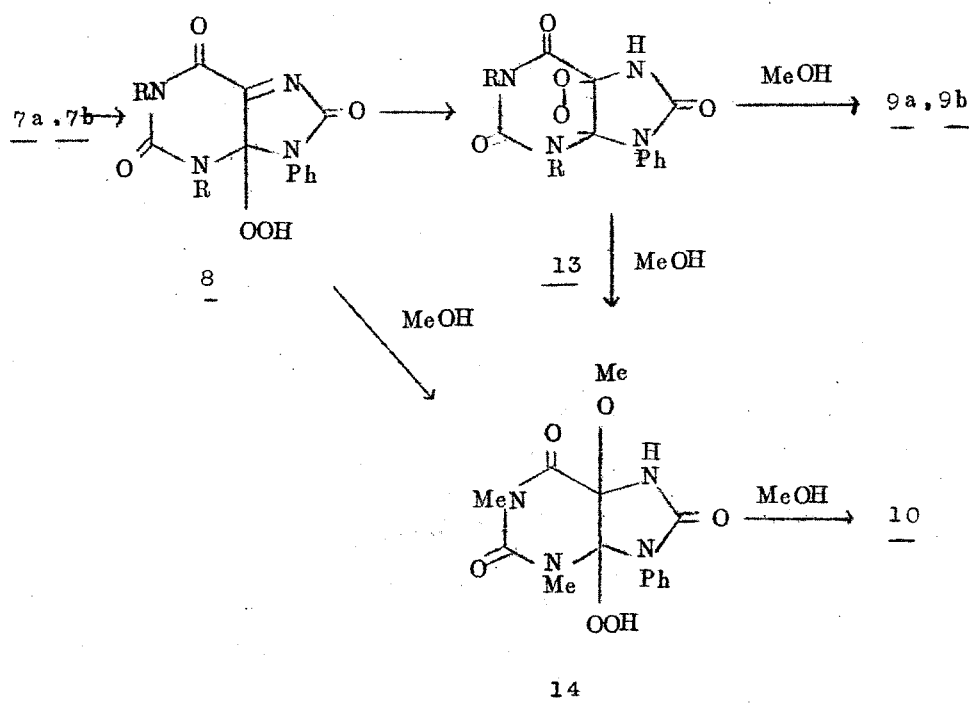
Although both 5 and 7 give rise to 9, different mechanisms must be considered for the formation of 9. As was expected, the formation of 9 from 5 can be rationalized by a 4,8-endo-peroxide intermediate 6. Such an endo-peroxide has been postulated as the intermediate in the photooxidation of some five-membered nitrogen heterocycles, i. e., pyrroles,<sup>7</sup> oxazoles,<sup>8</sup> and isoindoles.<sup>9</sup> The initially formed endo-peroxide 6 could be solvolyzed by methanol to

a methoxy-hydroperoxide 11 which is dehydrated to give a 8-keto compound 12. Such a process is well known in the photosensitized oxygenation of furan which, in methanol, gives 4-methoxy-2-butenolide via a 1,4-endo-peroxide.<sup>10</sup> As the result mentioned in the previous section, the formation of allantoin from xanthine (1a) provides an analogous example.<sup>1,2</sup> Addition of methanol to a C=N bond of 12 finally leads to the 4,5-dimethoxyuric acid derivative 9 (Scheme 1). A similar mechanism has been proposed for the photosensitized oxygenation of 2-methyl-5-phenyloxazole.<sup>8a</sup>



Scheme 1

On the other hand, a hydroperoxide 8 analogous to 4 can be considered to account for the formation of 9 from 7. This type of hydroperoxide is generally accepted as an intermediate in the photooxygenation of certain N-heterocycles bearing a  $-C=C-NH-$  moiety.<sup>1</sup> Hydroperoxide 8 could rearrange to a four-membered cyclic peroxide 13 which, in the presence of methanol undergoes reductive cleavage to give 9 (Scheme 2).



Scheme 2.

Such a process involving reductive cleavage of a cyclic peroxide by methanol to a dimethoxy compound has analogy in the photosensitized oxygenation of a cyclophane derivative.<sup>12</sup> A methoxy-hydroperoxide 14, which might be formed either from the cyclic peroxide 13 by methanolysis or directly from hydroperoxide 8 by addition of methanol to a C=N double bond, can account for the formation of 10 from 1,3-dimethyl-9-phenyluric acid (7a).

Recently involvement of singlet oxygen in dye-sensitized photooxygenation of olefinic compounds<sup>13</sup> including oxazoles<sup>14</sup> has been shown by several workers. In order to ascertain the participation of singlet oxygen in the present reactions, the reaction of 5b with chemically generated singlet oxygen was carried out. Treatment of 5b with hydrogen peroxide and sodium hypochlorite in an aqueous<sup>u</sup> methanolic solution at pH 8.4 afforded a complex mixture of products from which only a small amount of 4,5-dimethoxy-9-phenyluric acid (9b) was detected. The low yield of 9b is probably due to instability of 9b under conditions employed. At pH 8.4 5b was quite stable to hydrogen peroxide alone, but was oxidized with hypochlorite to give unidentified products

from which no 9b could be detected. However, it is probable that singlet oxygen is involved in the present photosensitized oxygenation.

### 3.3. Experimental

#### 3.3.1. 1,3-Dimethyl-9-phenylxanthine (5a).

To a suspension of 9-phenylxanthine<sup>15</sup> (1.0 g, 4.4 mmoles) in 100 ml of absolute MeOH was added an ether solution of diazomethane (prepared from 5g of nitrosomethylurea). After removal of the solvent, a solid insoluble in MeOH was again methylated in the similar manner. Following removal of the solvent the residue was extracted with  $\text{CHCl}_3$  and the extract was evaporated. Crystallization of the residue gave 0.13g (11.5%) of 5a, m.p. 305-307° (dec).  $\lambda_{\text{max}}^{\text{EtOH}}$  265 m $\mu$  ( $\epsilon$  21200),  $\nu_{\text{max}}^{\text{Nujol}}$  1690, 1640  $\text{cm}^{-1}$ , NMR ( $\text{CDCl}_3$ );  $\tau$

2.45-2.86 (5H, multiplet, phenyl), 2.50 (1H, singlet, -CH=N-), 6.65 (3H, singlet, >N-CH<sub>3</sub>), 6.98 (3H, singlet, >N-CH<sub>3</sub>). (Found: C, 60.46; H, 4.85; N, 21.41.  $\text{C}_{13}\text{H}_{12}\text{N}_4\text{O}_2$  requires C, 60.93; H, 4.72; N, 21.87%).

3.3.2. Photosensitized Oxygenation of 1,3-Dimethyl-9-phenylxanthine (5a).

A solution of 100 mg (0.4 mmole) of 5a and rose bengal (10 mg) in 100 ml of absolute MeOH was irradiated with a 100 W high-pressure mercury lamp for 1 hr as described previously, until 12 ml (0.50 mmole) of oxygen was consumed. After removal of the solvent a residual oil was purified by preparative TLC on silica gel using a mixture of  $\text{CHCl}_3$  and EtOH (20:1). The zone of Rf-value 0.30 was collected and eluted with acetone. From the eluate 30 mg (23%) of 1,3-dimethyl-4,5-dimethoxy-9-phenyluric acid (9a) was obtained as crystals. Recrystallization from MeOH gave fine needles, m.p. 223-225°, which were identical with an authentic sample prepared below (by mixture m.p. and IR).  $\lambda_{\text{max}}^{\text{EtOH}}$  220 m $\mu$  ( $\epsilon$  29800), 275 m $\mu$  ( $\epsilon$  1500).  $\nu_{\text{max}}^{\text{Nujol}}$  1720, 1675  $\text{cm}^{-1}$ . (Found: C, 53.55; H, 5.41; N, 16.69.  $\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_5$  requires C, 53.88; H, 5.43; N, 16.76%).

3.3.3. Reduction of 1,3-Dimethyl-4,5-dimethoxy-9-phenyluric Acid (9a) with HI.

The reduction of 9a was carried out according to the method of Biltz.<sup>16</sup> A solution of 150 mg (0.45 mmole)

of 9a obtained above in conc HI (5 ml) was warmed at 60° for 3 hr. Following removal of the solvent in vacuo, the residual oil was triturated with MeOH(20 ml) under ice-cooling to deposit 65 mg (53%) of 1,3-dimethyl-9-phenyluric acid (7a) as crystals, m.p. 300°, which were identified by IR.

#### 3.3.4. 1,3-Dimethyl-4,5-dimethoxy-9-phenyluric Acid(9a)

According to the procedure reported by Biltz,<sup>6</sup> a suspension of 1.0 g (3.7 mmoles) of 1,3-dimethyl-9-phenyluric acid<sup>17</sup> (7a) in 20 ml of absolute MeOH was cooled on a ice-salt bath. Chlorine gas was bubbled through the suspension yielding a clear solution. In order to remove the excess of chlorine a stream of nitrogen was bubbled through the solution, depositing white precipitates. Crystallization from MeOH gave 0.61 g(50%) of 9a as fine needles, m.p. 223-225°.

#### 3.3.5. 1,3,7-Trimethyl-4,5-dimethoxy-9-phenyluric Acid.

According to the above procedure, this compound was synthesized from 1,3,7-trimethyl-9-phenyluric acid<sup>18</sup> in 49% yield. Recrystallization from MeOH gave fine

needles, m.p. 138°.  $\lambda_{\text{max}}^{\text{EtOH}}$  221 m $\mu$  ( $\epsilon$  35600), 275 m $\mu$  ( $\epsilon$  2400),  $\nu_{\text{max}}^{\text{Nujol}}$  1740, 1680 cm<sup>-1</sup>, NMR (CDCl<sub>3</sub>);  $\tau$  2.87 (5H, multiplet, phenyl), 6.55, 6.61, 6.80. 7.17, 7.45 (all singlet, each 3H, >N-CH<sub>3</sub> and -O-CH<sub>3</sub>). (Found: C, 55.31; H, 5.64; N, 15.96. C<sub>16</sub>H<sub>20</sub>N<sub>4</sub>O<sub>5</sub> requires C, 55.16; H, 5.79; N, 16.08%).

This compound was also obtained from 1,3-dimethyl-4,5-dimethoxyuric acid (9a) by treatment with ethereal diazomethane in 55% yield.

### 3.3.6. Photosensitized Oxygenation of 9-Phenylxanthine (5b).

A suspension of 2.0 g (8.8 mmoles) of 5b in absolute MeOH (400 ml) containing rose bengal (20 mg) was irradiated with a 100 W high-pressure mercury lamp for 3 hr as described above. During irradiation carbon dioxide liberated was trapped as barium carbonate (0.73 g, 2.5 mmoles). After 232 ml (9.7 mmoles) of oxygen had been consumed, the mixture was concentrated in vacuo to 10 ml, then treated with Norit to remove the sensitizer. On cooling in an ice box overnight 0.52 g of 4,5-dimethoxy-9-phenyluric acid (9b) was deposited as crystals. The mother liquor was evaporat-



ed and the residue was chromatographed on a silica gel column (40 g). Elution with  $\text{CHCl}_3$ -acetone (10:1) yielded 1.05 g (total yield, 58%) of 9b. Recrystallization from MeOH gave crystals, m.p. 190-191°.  $\lambda_{\text{max}}^{\text{EtOH}}$  218 m $\mu$  ( $\epsilon$  36000), 248 (shoulder, 18800),  $\nu_{\text{max}}^{\text{Nujol}}$  1770, 1670  $\text{cm}^{-1}$ . (Found: C, 50.54; H, 4.82; N, 17.81.  $\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}_5$  requires C, 50.98; H, 4.61; N, 18.28%).

### 3.3.7. Methylation of 4,5-Dimethoxy-9-phenyluric Acid (9b).

An excess of ethereal diazomethane was added to a solution of 9b (100 mg). After removal of the solvent, the residue was again treated with ethereal diazomethane. The mixture was found by TLC to consist of several products, from which 5 mg of 1,3-dimethyl-4,5-dimethoxy-9-phenyluric acid (9a) was isolated by preparative TLC using silica gel plates and  $\text{CHCl}_3$ -EtOH (10:1) as an eluting solvent, and the compound was identical with a sample obtained above (by IR).

### 3.3.8. Photosensitized Oxygenation of 1,3-Dimethyl-9-phenyluric Acid (7a).

A suspension of 1.0 g (3.7 mmol) of 7a in 280

ml of MeOH containing 20 mg of rose bengal was photo-oxidized as described above (photooxidation of 5a). After 95 ml (4.0 mmoles) of oxygen had been consumed, the mixture was evaporated. The residue was chromatographed on a silica gel column (20 g). Elution with 200 ml of  $\text{CHCl}_3$  yielded 25 mg (2.1%) of 1,3-dimethyl-4,5-dimethoxy-9-phenyluric acid (9a) which was identified by IR. Elution with  $\text{CHCl}_3$ -acetone (20:1) yielded 132 mg (12%) of 1,3-dimethyl-4-hydroxy-5-methoxy-9-phenyluric acid (10) as crystals. Recrystallization from acetone gave crystals, m.p. 201-202°.  $\lambda_{\text{max}}^{\text{EtOH}}$  232 m $\mu$  ( $\epsilon$  27700) and 271 (1700);  $\nu_{\text{max}}^{\text{Nujol}}$  3300-3400, 1750 (shoulder), 1690, and 1655  $\text{cm}^{-1}$ . (Found: C, 52.50; H, 4.96; N, 17.51.  $\text{C}_{14}\text{H}_{16}\text{N}_4\text{O}_5$  requires C, 52.49; H, 5.04; N, 17.49%).

### 3.3.9. Partial Hydrolysis of 1,3-Dimethyl-4,5-dimethoxy-9-phenyluric Acid (9a).

A suspension of 100 mg (0.3 mmole) of 9a in 3 ml of 2N HCl was boiled for 1 min. After cooling a solid deposited was collected by filtration and crystallized from acetone to give 15 mg (15%) of 1,3-dimethyl-4-

hydroxy-5-methoxy-9-phenyluric acid (10) as crystals, m.p. 200-202<sup>o</sup>, which were identical with the acid 10 obtained from 7a (by IR).

3.3.10. Photosensitized Oxygenation of 9-Phenyluric Acid (7b).

A suspension of 1.5 g (6.3 mmoles) of 9-phenyluric acid (7b)<sup>18</sup> in 200 ml of absolute MeOH containing 20 mg of rose bengal was photooxidized for 4 hr as described above. After 180 ml (7.1 mmoles) of oxygen had been consumed, the mixture was evaporated in vacuo to 15 ml. Crystals deposited (0.86 g, 46%) were identified as 4,5-dimethoxy-9-phenyluric acid (9b) (by IR and mixture m.p.).

3.3.11. Oxidation of 9-Phenylxanthine (5b) with Hydrogen Peroxide and Sodium Hypochlorite.

The oxidation was carried out by the procedure reported by Foote et al.<sup>13</sup> To a solution of 1.0 g (4.4 mmoles) of 5b in 300 ml of MeOH was added 2.5 ml (22.5 mmoles) of 30% H<sub>2</sub>O<sub>2</sub>. The mixture was cooled on an ice-bath and 14 ml (17 mmoles) of 9% NaOCl aq was added dropwise under stirring in the course of

1 hr. To the reaction mixture (pH 8.4) was added 500 ml of  $\text{CHCl}_3$  and 500 ml of water. The organic layer was separated and evaporated in vacuo to dryness. The residue was found by TLC to consist of at least seven compounds. One of the compounds was identified by TLC (silica gel,  $\text{CHCl}_3$ -EtOH (10:1)) and by PPC (n-BuOH-AcOH- $\text{H}_2\text{O}$  (5:4:1)) as 4,5-dimethoxy-9-phenyluric acid (9b).

At the same pH (8.4) 5b was recovered unchanged on treatment with hydrogen peroxide. Reaction of 5b with an excess of NaOCl was also carried out. To a solution of 100 mg (0.44 mmole) of 5b in 100 ml of MeOH 5 ml (6 mmoles) of 9% NaOCl aq was added. The mixture was extracted with  $\text{CHCl}_3$  as described above. The  $\text{CHCl}_3$  extract was found by TLC to consist of mainly two products, which were not identical with 4,5-dimethoxy-9-phenyluric acid (9b).

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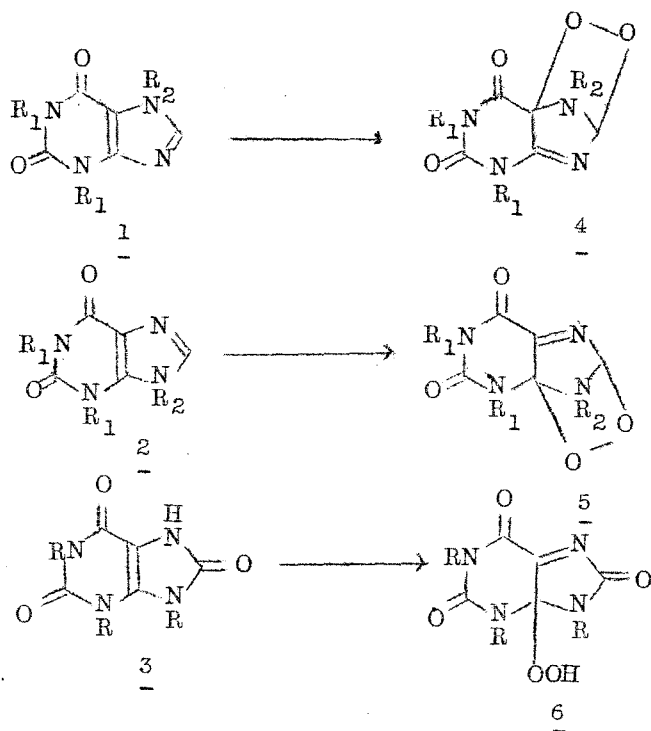
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## 4. Photosensitized Oxygenation of Fully N-Alkylated Uric Acids

### 4.1. Introduction

In the previous section,<sup>1,2,3</sup> it has been shown that, in the case of 2,6-dihydroxypurine derivatives such as 1, 2, and 3, the initial attack of oxygen, possibly excited singlet oxygen, occurs at double bonds of the imidazole moiety to form a peroxide intermediate which could account for the formation of the degradation products. Two types of peroxide intermediates have been formulated in these reactions. When two double bonds are present in the imidazole moiety as in 1 and 2, an endo-peroxide as 4 and 5 might be the intermediate. It is generally accepted that such an endo-peroxide is an intermediate in the photosensitized oxygenation of cyclic dienes<sup>4</sup> and also of certain N-heterocycles<sup>5,6</sup> bearing a -C=N-C=C- group. On the other hand, when a -C=C-NH- group is present in the imidazole moiety as in 3, a hydroperoxide as 6 might be formed being accompanied by migration of the double bond. This type of hydro-

peroxide is also generally accepted as an intermediate in the photooxygenation of various N-heterocycles such as imidazoles<sup>6</sup>, pyrroles<sup>7</sup>, and indoles.<sup>8</sup>



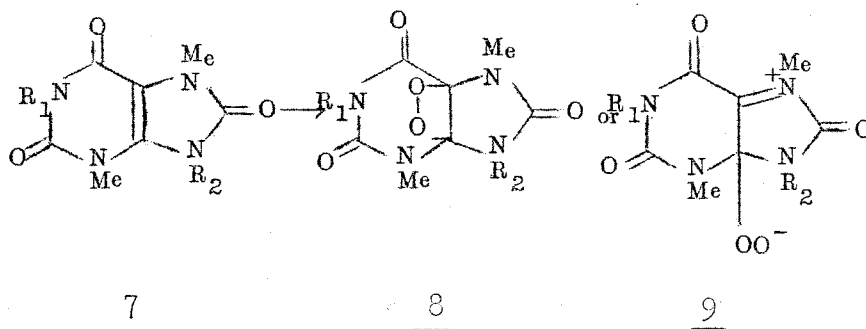
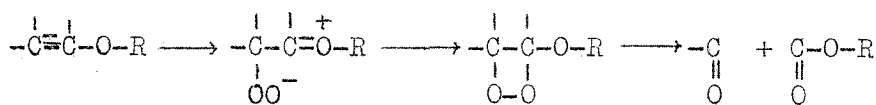
The latter type of peroxide is well known as an initial product in the photooxygenation of simple olefins bearing an allylic hydrogen; namely  $-C=C-CH-$   $\longrightarrow$   $-C(OOH)-C=C-$ . To the best of our knowledge, however, photosensitized oxygenation of olefins having no allylic hydrogen has not been reported, although some compounds



having a vinyl ether group,  $-C=C-O-R$ , without having any allylic hydrogen undergo photooxidative cleavage of  $C=C$  double bond.<sup>9\*</sup> It appeared, therefore, interesting to investigate photosensitized oxygenation of fully N-alkylated uric acids (7) which have no NH group in contrast to 3. From a compound such as 7 it might be expected to form a four-membered cyclic peroxide (8) or a zwitterionic peroxide (9).

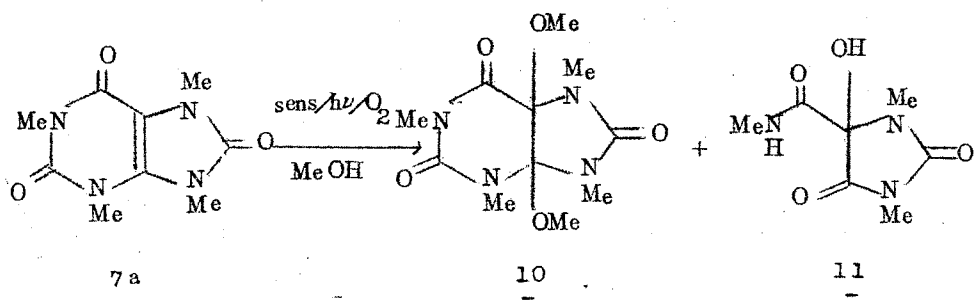
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\* It has been proposed that cleavage of  $C=C$  double bond proceeds via a four-membered cyclic peroxide intermediate.<sup>9b</sup> However, a zwitterionic peroxide intermediate may also account for the cleavage reaction (see below) as follows.



#### 4.2. Results and Discussion

When a methanol solution of 1,3,7,9-tetramethyluric acid (7a) was submitted to photosensitized oxygenation in the presence of rose bengal, 0.63 mole of oxygen was consumed rapidly and 4,5-dimethoxy-1,3,7,9-tetramethyluric acid (10) and allacaffeic acid (11) were obtained in 35 and 5% yield, respectively. Both products were identified by their independent syntheses.<sup>10,11</sup> Allocaffeic acid (11) is considered to be formed by hydrolysis of 10 in the course of the chromatographic separation of the products. In fact, 10 is easily hydrolyzed by acid treatment to give 11.<sup>11</sup>



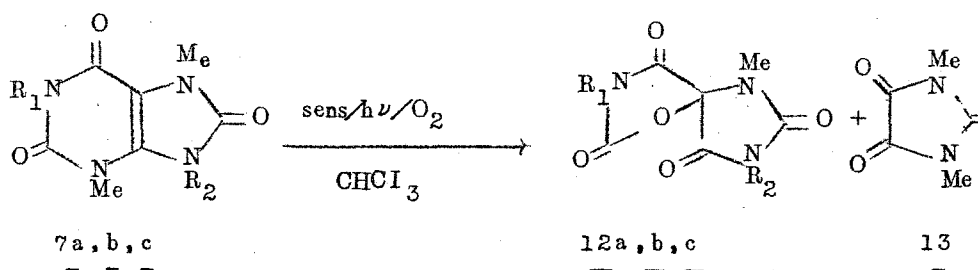
On the other hand, when photosensitized oxygenation was carried out in chloroform using methylene blue as sensitizer, fully N-alkylated uric acids (7) gave

different types of products. Thus, 1,3,7,9-tetramethyluric acid (7a) afforded, after absorption of 0.59 mole of oxygen with a slower rate than in methanol, 1,3,7-trimethylcaffolide (12a)<sup>11</sup> and 1,3-dimethylparabanic acid (13) in 42 and 7% yield, respectively. Both compounds were identified by a direct comparison with authentic specimens.

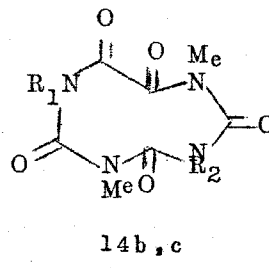
Under the same conditions, 1-ethyl-3,7,9-trimethyluric acid (7b) absorbed 0.72 mole of oxygen in chloroform to yield 1,3-dimethyl-7-ethyl-caffolide (12b),<sup>12</sup> 1,3-dimethylparabanic acid (13), and an oxygen adduct  $C_{10}H_{14}N_4O_5$  in 12, 8, and 22% yield, respectively. The structure of 12b was confirmed by its synthesis and by its hydrolysis to 1,3-dimethyl-5-hydroxyhydantoin-5-N-ethylcarboxamide.<sup>12</sup> Molecular formula for the adduct was confirmed by the appearance of its parent peak at  $m/e$  270. The IR spectrum shows a broad carbonyl absorption band at  $1750-1650\text{ cm}^{-1}$  but neither NH or OH band. The NMR spectrum exhibits three singlets at  $\tau$  6.85, 7.01, and 7.10 corresponding to three N-methyl groups, a quartet at  $\tau$  6.32 ( $J = 7\text{ cps}$ ), and a triplet at  $\tau$  8.87 ( $J = 7\text{ cps}$ ).

The latter two signals were assigned to an N-ethyl group. Hydrolysis of the adduct with boiling water afforded 1,3-dimethyl- and 1-ethyl-3-methylparabanic acids in 67 and 26% yields, respectively. The above results led us to assign structure 14b for this compound.

Photosensitized oxygenation of 9-ethyl-1,3,7-trimethyluric acid (7c) in chloroform yielded 1,7-dimethyl-3-ethylcaffolide (12c) and an oxygen adduct (14c) in 5 and 16% yield, respectively, after absorption of 0.64 mole of oxygen. The former product was identified by a direct comparison with an authentic sample.<sup>13</sup> The spectral data of the oxygen adduct are quite analogous to those of the adduct 14b, and structure 14c was given.



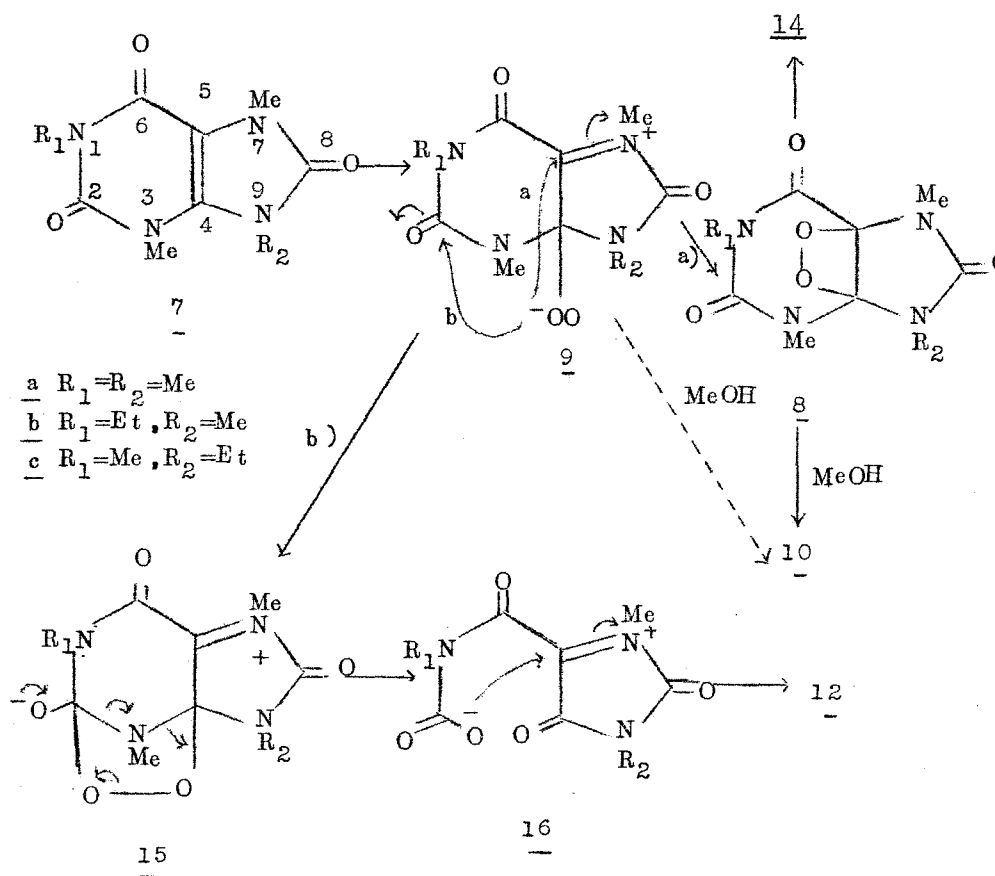
- a  $\text{R}_1 = \text{R}_2 = \text{Me}$   
b  $\text{R}_1 = \text{Et}, \text{R}_2 = \text{Me}$   
c  $\text{R}_1 = \text{Me}, \text{R}_2 = \text{Et}$



Although the formation of 4,5-dimethoxy-1,3,7,9-tetramethyluric acid (10) from 7a looks quite analogous to the conversion of 1,3-dimethyl-9-phenyluric acids (3) to corresponding 4,5-dimethoxyuric acid derivatives, previously mentioned in section 3 of Chapter I, the hydroperoxide intermediate such as 6 can not account for the transformation of 7a into 10 because of the lack of C=C-NH group as in 3. It is, therefore, proposed a zwitterionic peroxide 9a for the first step intermediate in the photosensitized oxygenation of 7a. As was discussed above, the four-membered cyclic peroxide 8a could also account for the formation of 10. However, the results obtained in the photosensitized oxygenation of 7a, 7b and 7c in chloroform are strongly supported the initial formation of the zwitterionic peroxide 9. The transformation of 7 into 1,3,7-trialkylcaffolides 12 clearly indicates that the 3-N-methyl group was expelled in the course of the formation of 12.

The decomposition process of the zwitterionic peroxide 9 depends upon its surroundings. As shown in Scheme 1, 9 could rearrange to the cyclic peroxide

8 (path a) which, in the presence of methanol, undergoes reductive cleavage to form the dimethoxyuric acid 10. An alternative route, by which the zwitterion 9a is reductively solvolyzed with methanol at 4-position being accompanying by an addition of methanol to the C=N bond, can also be considered. 4,5-Bond fission leading to the medium ring compounds 14b and 14c may occur either from 8 stepwise via 9 or directly from 9 by a concerted process. Finally, the formation of caffolides 12 could be rationalized only by the zwitterionic intermediate 9. A cyclic tautomer 15, which is resulted from a nucleophilic attack of the perhydroxy anion to a carbonyl carbon at 2-position (path b), could lose 3-N-methyl group to form an intermediate 16 which in turn cyclizes to 12. The lose of the 3-N-methyl group has analogy in the photosensitized oxygenation of 1,3-diphenyl-2-methylisoindole leading to o-dibenzoylbenzene.<sup>14</sup> Zwitterionic peroxides such as 9 have been proposed by Foote et al<sup>15</sup> and Wasserman et al<sup>16</sup> as the intermediate of the photosensitized oxygenation of a number of fully N-substituted enamines.



Scheme 1

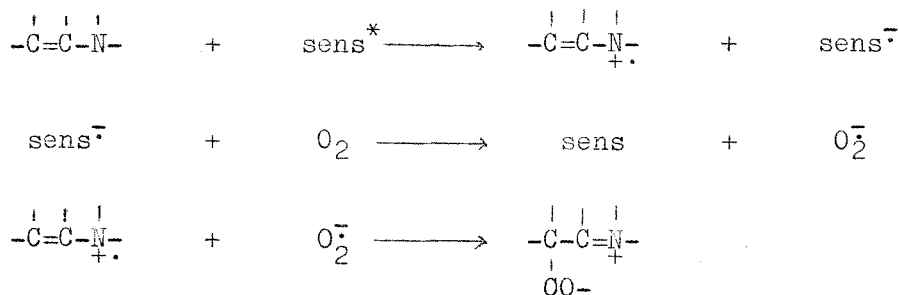
Evidences for the participation of singlet oxygen in dye-sensitized photooxygenation have been provided by various workers.<sup>17-20</sup> In order to elucidate whether singlet oxygen is involved in the photosensitized oxygenation of 7a, 7b and 7c, reaction of 7a and 7b with singlet oxygen, which was generated

by non-photochemical means, was investigated. Reaction of 7a with hydrogen peroxide and hypochlorite in aqueous methanol<sup>17</sup> afforded 4,5-dimethoxy -1,3,7,9-tetramethyluric acid (10) and 1,3-dimethylparabanic acid (13) in 2 and 38% yield, respectively. The latter product seems to be formed by hydrolytic decomposition of 10. In control experiments, 7a was completely intact to hydrogen peroxide alone, but it was decomposed by hypochlorite in aqueous methanol to give various products among which 10 and 13 could not be detected. Reaction of 7b with 9,10-diphenylanthracene peroxide in boiling chloroform<sup>18</sup> gave, in addition to recovery of a bulk of the starting material, only a trace of 1,3-dimethyl-7-ethylcaffolide (12b) which was detected by TLC on silica gel. On the other hand, when 7a was treated with an excess of triphenyl phosphite-ozone adduct at -30<sup>o</sup><sup>19</sup>, 1,3,7-trimethyl-caffolide (12a) was obtained in 5% yield.

The above results indicate that the zwitterionic peroxides (9) may be formed at least in part by the attack of singlet oxygen to the tetraalkyluric acids (7) in the photosensitized oxygenation. An alternative pathway involving an electron transfer from a substrate



to the triplet excited sensitizer may also account for the formation of the zwitterionic peroxide, as equations shown below. Such an electron transfer mechanism has been postulated by Morita and Kato



from the flash photolysis experiments on guanine in the presence of thionine.<sup>21</sup> Although, from the available data, it cannot be rigorously distinguished between these two mechanisms for the formation of the zwitterionic peroxide, the direct attack of singlet oxygen appears to be more favorable in view of previous reports on the electrophilic properties of singlet oxygen.<sup>22,23</sup>

#### 4.3. Experimental

##### 4.3.1. Photosensitized Oxygenation of 1,3,7,9-Tetramethyluric Acid (7a).

###### A. In Methanol

A solution of 1,3,7,9-tetramethyluric acid<sup>24</sup> (7a) (2.0 g, 8.90 mmoles) in a mixture of abs. MeOH (100 ml) and CHCl<sub>3</sub> (5 ml) containing rose bengal (20 mg) was irradiated by a tungsten lamp for 1 hr as described previously,<sup>3</sup> until oxygen (140 ml, 5.6 mmoles) was consumed. The mixture was evaporated and the residue was chromatographed on a silica gel column (40 g). Elution with CHCl<sub>3</sub> (ca. 100 ml) yielded 4,5-dimethoxy-1,3,7,9-tetramethyluric acid (10) as a crystalline solid (0.87 g, 35%). Recrystallization from acetone gave crystals, m.p. 127-128° (lit.<sup>10</sup>, m.p. 133°), which were identical with an authentic sample prepared according to the method of Biltz et al<sup>10</sup> (by IR).  $\nu_{\text{max}}^{\text{Nujol}}$  1730 and 1680 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>),  $\delta$  6.61, 6.68, 6.80, 6.98, 7.10, and 7.21 (all singlets, >N-Me and -OMe).

Further elution with CHCl<sub>3</sub>-acetone (97:3) gave allocaffeic acid (11) as a semisolid (85 mg, 5%). Recrystallization from ethyl acetate gave crystals, m.p. 170° (lit.,<sup>11</sup> m.p. 168-169°). The compound was identical with the authentic sample prepared according to the method of Biltz<sup>11</sup> (by IR).  $\nu_{\text{max}}^{\text{Nujol}}$  1790, 1730, and 1665 cm<sup>-1</sup>; NMR (DMSO-d<sub>6</sub>),  $\delta$  1.80 (singlet, 1H, NH),

2.42 (singlet, 1H, OH), 7.41 (singlet, 3H, >N-Me), 7.45 (doublet ( $J = 5\text{cps}$ ), 3H, -NH-Me), 7.18 (singlet, 3H, >N-Me).

When a 100 W high-pressure mercury lamp through a Pyrex filter was used in the photosensitized oxygenation, the results were virtually the same as above.

B. In  $\text{CHCl}_3$

A solution of 7a (2.0 g, 8.90 mmoles) in  $\text{CHCl}_3$  (100 ml) containing methylene blue (50 mg) was irradiated by a 100 W high-pressure mercury lamp for 4 hr, until oxygen (130 ml, 5.2 mmoles) was consumed. After removal of the solvent, the residue was chromatographed on a silica gel column (40 g). Elution with  $\text{CHCl}_3$  (ca. 300 ml) gave 1,3,7-trimethylcaffolide (12a) as a crystalline solid (0.85 g, 42%). Recrystallization from EtOH yielded crystals, m.p.  $207-208^\circ$  (lit.<sup>11</sup>, m.p.  $205^\circ$ ), which were identical with the authentic specimen prepared according to the procedure of Biltz<sup>11</sup> (by IR).  $\nu_{\text{max}}^{\text{Nujol}}$  1800, 1760-1690  $\text{cm}^{-1}$ ; NMR ( $\text{CHCl}_3$ ),  $\tau$  6.84 (singlet, 3H, >N-Me), 6.96 (singlet, 3H, >N-Me), 7.14 (singlet, 3H, >N-Me).

Further elution with  $\text{CHCl}_3$  gave 1,3-dimethylparabanic acid (13) (95 mg, 7%), identified by IR.

4.3.2. Photosensitized Oxygenation of 1-Ethyl-3,7,9-trimethyluric Acid (7b),

A solution of 1-ethyl-3,7,9-trimethyluric acid<sup>12</sup> (7b) (2.0 g, 8.4 mmoles) and methylene blue (50 mg) in  $\text{CHCl}_3$  (100 ml) was irradiated by a 100 W high-pressure mercury lamp for 2.5 hr, until oxygen (150 ml, 6.0 mmoles) was consumed. After removal of the solvent, the residue was chromatographed on a silica gel column (30 g). Elution with  $\text{CHCl}_3$  (100 ml) gave the oxygen adduct 14b as a crystalline solid (0.51 g, 22%). Recrystallization from acetone gave crystals, m.p. 101-103°.  $\epsilon_{210}^{\text{EtOH}}$  37,200;  $\nu_{\text{max}}^{\text{Nujol}}$  1750-1710, 1670  $\text{cm}^{-1}$ ; mass spectrum (m/e), 270 (parent peak), 241, 226, 212, 196, 185, 170, 156, 143 (base peak). (Found: C, 44.27; H, 5.15; N, 20.15.

$\text{C}_{10}\text{H}_{14}\text{N}_4\text{O}_5$  requires: C, 44.44; H, 5.22; N, 20.73%).

Elution with  $\text{CHCl}_3$  (250 ml) yielded 1,3-dimethyl-7-ethylcaffolide (12b) (0.22 g, 12%). Recrystallization from EtOH gave crystals, m.p. 95-96° (lit.,<sup>12</sup> m.p. 93°), which were identical with the authentic sample

prepared according to the method of Biltz et al<sup>12</sup>.  
(by mixture m.p. and IR).  $\nu_{\text{max}}^{\text{Nujol}}$  1800, 1730  $\text{cm}^{-1}$ ;  
NMR ( $\text{CDCl}_3$ ),  $\tau$  6.98 (singlet, 3H,  $\gamma$ -N-Me), 7.16 (singlet,  
3H,  $\gamma$ -N-Me), 6.39 (quartet ( $J = 7$  cps), 2H,  $\gamma$ -N- $\text{CH}_2$ -Me),  
8.71 (triplet ( $J = 7$  cps), 3H,  $\gamma$ -N- $\text{CH}_2$ -Me).

Elution with  $\text{CHCl}_3$  (400 ml) gave 1,3-dimethyl-  
parabanic acid (13) (0.11 g, 8%), identified by IR.

#### 4.3.3. Hydrolysis of 14b

A suspension of 14b (50 mg) in  $\text{H}_2\text{O}$  (5 ml) was  
boiled for 3 hr. After removal of the water in vacuo,  
the residue was extracted with acetone (5 ml). The  
extract was purified by preparative thin layer  
chromatography on silica gel plates using  $\text{CHCl}_3$ -EtOH  
(20:1) as a solvent. Bands of Rf 0.70 and Rf 0.50  
were cut off and then extracted with acetone. From  
the band of Rf 0.70, 1-ethyl-3-methylparabanic acid  
(5 mg, 26%), m.p. 47-48° (lit., <sup>25</sup> m.p. 44°), was  
obtained. 1,3-Dimethylparabanic acid (18 mg, 67%)  
was obtained from the band of Rf 0.50. Both compounds  
were identified by IR.

4.3.4. Hydrolysis of 1,3-Dimethyl-7-ethylcaffolide (12b) to 1,3,-Dimethyl-5-hydroxyhydantoin-5-N-ethyl-carboxyamido.

1,3-Dimethyl-7-ethylcaffolide (12b) was hydrolyzed according to the method of Biltz and Max.<sup>12</sup> A suspension of 12b (0.5 g) in H<sub>2</sub>O (15 ml) was boiled for 30 min. After removal of the water, the residue was recrystallized from ethyl acetate to give 1,3-dimethyl-5-hydroxyhydantoin-5-N-ethylcarboxyamido (0.21 g, 68%), m.p. 156-157.5° (lit.,<sup>12</sup> m.p. 153°).  $\nu_{\text{max}}$  Nujol 1780, 1710, 1650 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>),  $\tau$  2.94 (singlet, 1H, NH) 3.93 (singlet, 1H, OH) 7.11 (singlet, 3H, > N-Me), 7.27 (singlet, 3H, > N-Me), 6.71 (quartet-doublet (J = 7cps, J = 4cps), 2H, -NH-CH<sub>2</sub>-Me), 8.85 (triplet (J = 7cps), 3H, -NH-CH<sub>2</sub>-Me).

4.3.5. Photosensitized Oxygenation of 9-Ethyl-1,3,7-trimethyluric Acid (7c).

A solution of 9-ethyl-1,3,7-trimethyluric acid (7c)<sup>24</sup> (2.0 g, 8.4 mmoles) in CHCl<sub>3</sub> (100 ml) containing methylene blue (50 mg) was photooxidized as described above for 12 hr, until oxygen (135 ml, 5.4 mmoles) was consumed. After removal of the solvent, the residue was chromato-

graphed on a silica gel column (40 g). Elution with  $\text{CHCl}_3$  (150 ml) gave 14c as a crystalline solid (0.21 g, 16%). Recrystallization from acetone yielded crystals, m.p.  $94-95^\circ$ ,  $\epsilon_{210}^{\text{EtOH}}$  38,100;  $\nu_{\text{max}}^{\text{Nujol}}$  1740-1700,  $1660\text{ cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ),  $\tau$  6.80 (singlet, 3H,  $\text{>NMe}$ ), 6.86 (singlet, 3H,  $\text{>N-Me}$ ), 6.96 (singlet, 3H,  $\text{>N-Me}$ ), 6.54 (quartet ( $J = 7\text{ cps}$ ), 2H,  $\text{>N-CH}_2\text{-Me}$ ) 8.96 (triplet ( $J = 7\text{ cps}$ ), 3H,  $\text{>N-CH}_2\text{-Me}$ ); mass spectr (m/e), 270 (parent peak). (Found: C, 44.39; H, 5.39 N, 20.28.  $\text{C}_{10}\text{H}_{14}\text{N}_4\text{O}_5$  requires: C, 44.44; H, 5.22; N, 20.73%).

Further elution with  $\text{CHCl}_3$  (300 ml) gave 1,7-dimethyl-3-ethylcaffolide (12c) (50 mg, 5%). Recrystallization from EtOH gave crystals, m.p.  $103-105^\circ$  (lit.,  $^{14}\text{ m.p. } 102^\circ$ ), which were identical with the authentic sample prepared according to the method of Biltz et al.<sup>13</sup>  $\nu_{\text{max}}^{\text{Nujol}}$  1800,  $1750-1700\text{ cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ),  $\tau$  6.88 (singlet, 3H,  $\text{>N-Me}$ ), 7.17 (singlet, 3H,  $\text{>N-Me}$ ) 6.47 (quartet ( $J = 7\text{ cps}$ ), 2H,  $\text{>N-CH}_2\text{-Me}$ ), 8.78 (triplet ( $J = 7\text{ cps}$ ), 3H,  $\text{>N-CH}_2\text{-Me}$ ).

Further elution with  $\text{CHCl}_3$  (500 ml) gave the starting material (7c) (0.85 g, 42%).

#### 4.3.6. Oxidation of 7a and 7b with Singlet Oxygen from Various Sources.

##### A. From Hydrogen Peroxide and Hypochlorite

The reaction was carried out according to the procedure of Foote et al.<sup>17</sup> A solution of 1,3,7,9-tetramethyluric acid (7a) (1.0 g, 4.45 mmoles) in MeOH (300 ml) was cooled on an ice-bath, and 30% H<sub>2</sub>O<sub>2</sub> (5 ml, 45 mmoles) was added. To the mixture 9% NaOCl aq (28 ml, 33 mmoles) was added dropwise under stirring in a period of 1 hr. The reaction mixture (pH 8.9) was diluted with H<sub>2</sub>O (500 ml) and extracted with CHCl<sub>3</sub> (500 ml). The extract was chromatographed on a silica gel column (20 g). Elution with CHCl<sub>3</sub> gave 1,3-dimethylparabanic acid (13) (60 mg, 38%), 4,5-dimethoxy-1,3,7,9-tetramethyluric acid (10) (5 mg, 2%) and the unreacted starting material (7a) (0.75 g, 75% recovery). These products were identified by IR.

To a solution of 7a (100 mg) in MeOH (30 ml) 30% H<sub>2</sub>O<sub>2</sub> (0.5 ml) was added at room temperature, and the reaction mixture was stirred for 1 hr. The starting material (7a) was recovered quantitatively.

To a solution of 7a (100 mg) in MeOH (30 ml) 9%



NaOCl aq (3 ml) was added as described above. The reaction mixture was found by TLC to consist of the unreacted starting material (7a) and three unidentified compounds, among which 10 and 13 could not be detected.

B. From 9,10-Diphenylanthracene Peroxide.

The reaction was carried out according to the procedure of Wasserman et al.<sup>18</sup> A solution of 1-ethyl-3,7,9-trimethyluric acid (7b) (100 mg, 0.42 mmole) and 9,10-diphenylanthracene peroxide (300 mg, 0.83 mmole) in  $\text{CHCl}_3$  (20 ml) was boiled for 3 days. A TLC analysis (silica gel,  $\text{CHCl}_3$ -EtOH (20:1)) of the reaction mixture showed the presence of a trace of 1,3-dimethyl-7-ethylcaffolide (12b) and a bulk of the unreacted starting material. The starting material (7b) was recovered over 90%.

C. From the Triphenyl Phosphite - Ozone Adduct.

According to the procedure of Thompson<sup>26</sup> and Kaplan<sup>19</sup>, the triphenyl phosphite-ozone adduct was prepared from triphenyl phosphite (6.0 g, 17.0 mmoles) in  $\text{CH}_2\text{Cl}_2$  (200 ml). To the solution a cold solution of 7a (2.0 g, 8.9 mmoles) in  $\text{CH}_2\text{Cl}_2$  (50 ml) was

added, and the reaction mixture was allowed to warm slowly to  $-30^{\circ}$  in a period of 8 hr and then to room temperature. The mixture was found by TLC to consist of at least six compounds along with triphenyl phosphate. After removal of the solvent, the residue was chromatographed on a silica gel column (65 g). Elution with  $\text{CHCl}_3$  (150 ml) yielded triphenyl phosphate (5.3 g, 85%). Further elution with  $\text{CHCl}_3$  (500 ml) gave 1,3,7-trimethylcaffolide (12a) (85 mg, 5% based on the reacted 7a). Recrystallization from EtOH gave crystals, m.p.  $205-207^{\circ}$ , which were identical with the authentic sample described above (by IR). Elution with  $\text{CHCl}_3$  (600 ml) yielded the unreacted starting material (7a) (0.18 g, 9% recovery). Acetone eluted red brown polymers (0.88 g).

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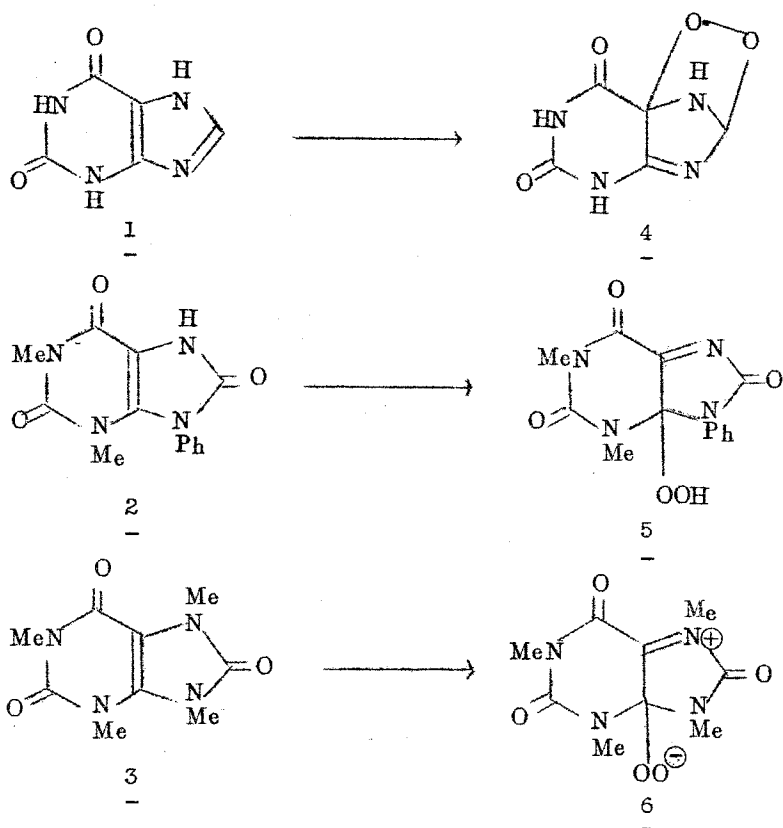
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## 5. Photosensitized Oxygenation of 8-Alkoxycaffeines and Related Compounds

### 5.1. Introduction

In the previous section<sup>1</sup>, the photosensitized oxygenation of various types of purine derivatives, including xanthine (1)<sup>2</sup>, 1,3-dimethyl-9-phenyluric acid (2)<sup>3</sup>, and 1,3,7,9-tetramethyluric acid (3)<sup>3</sup> have been carried out. To account for the products obtained from these reactions it was proposed that the reaction proceeds via a peroxide intermediate which may be formed by the attack of singlet oxygen to the substrate. The nature of the peroxide is depending upon the structural feature of the imidazole moiety of the purines. Thus, 1, 2, and 3 give a cyclic peroxide 4, a hydroperoxide 5, and a zwitterionic peroxide 6, respectively. In this section the results on the photosensitized oxygenation of 8-alkoxycaffeines and related compounds, which have provided further information concerning with structure of the peroxide intermediate and with its decomposition mode, are described.



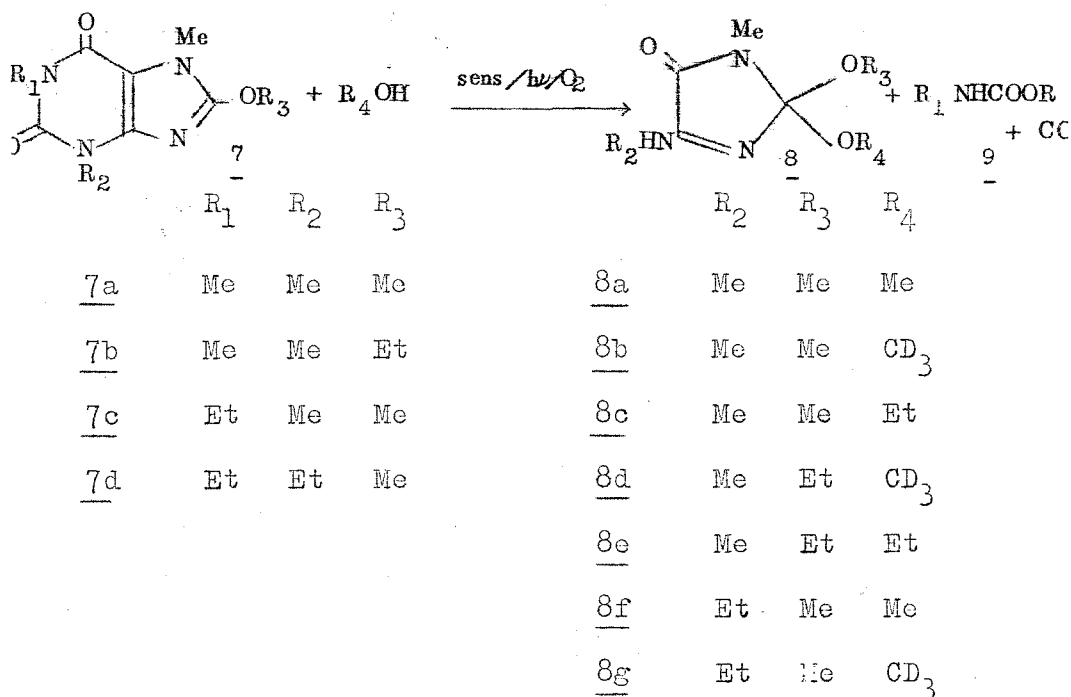
Although caffeine easily suffers photosensitized oxygenation in alkaline media<sup>4</sup>, it does not undergo degradation in a neutral organic solvent such as methanol and chloroform. However, 8-alkoxycaffeines are very sensitive to photosensitized oxygenation even in methanol. This is consistent with the electrophillic character<sup>5</sup> of singlet oxygen which is regarded as the reactive species in dye-sensitized photooxygenation<sup>6</sup>. Substitution of an electron donating alkoxy group to

the 8-position of caffeine may cause an increase of electron densities in the system. A similar effect has been observed in the photooxygenation of 1,4-dimethoxy-9,10-diphenylanthracene which does not give an usual 9,10-endo-peroxide but only a 1,4-endo-peroxide<sup>7</sup>.

## 5.2. Results and Discussion

When a solution of 8-methoxycaffeine (7a) in methanol-chloroform (20:1) was irradiated in the presence of rose bengal under bubbling oxygen, 0.8 mole of oxygen was consumed and liberation of 0.6 mole of carbon dioxide was observed. From the reaction mixture a crystalline product,  $C_{13}H_{13}N_3O_3$ , was obtained in 78% yield. Its UV spectrum shows a maximum at 218 m $\mu$  ( $\epsilon$  28000) with a shoulder at 252 m $\mu$  ( $\epsilon$  6200), and the IR spectrum shows bands at 3350 (NH), 1750, and 1665  $cm^{-1}$ . The NMR spectrum (Table 1) suggests the presence of -NH-Me, >N-Me, and two equivalent -O-Me groups. These spectral data are compatible with structure 8a for the product. In order to ascertain this structure, it should be clarified follow-

ing three questions; (i) whether one of the 2-methoxy groups of 8a comes from the solvent methanol, (ii) which N-methyl group of 7a is expelled in the course of the reaction, and (iii) which N-methyl group of 7a is converted to the 4-methylamino group in 8a. The structural assignment for 8a was confirmed by results obtained in the photosensitized oxygenation of various N-alkylated 8-alkoxyxanthines. The results are summarized in Table 1 which includes also the NMR *data* of the products.





Photosensitized oxygenation of 7a in methanol-d<sub>4</sub> and in ethanol yielded the corresponding products 8b (28%) and 8c (33%), respectively. The NMR spectra of 8b and 8c are virtually the same as that of 8a except that one of the two equivalent methoxy groups ( $\tau$  6.80) of 8a is replaced by a methoxy-d<sub>3</sub> group in 8b and by an ethoxy group in 8c. It is, therefore, obvious that the solvent alcohol has been incorporated into one of the two alkoxy groups of 8a, 8b, and 8c. From above results and the fact that the two methoxy groups of 8a is magnetically equivalent, it could be suggested that the alkoxy group incorporated from the solvent alcohol is introduced at the same position as the 8-methoxy group being originally present in 7a, provided that a drastic skeletal change of the imidazole moiety of 7a does not occur in the course of the reaction. This was further supported by the following experiments.

Photooxygenation of 8-ethoxycaffeine (7b) in methanol yielded a product (71%) which was identical with 8c. Under similar conditions 7b afforded 8d (24%) in methanol-d<sub>4</sub> and 8e (25%) in ethanol. The

Table 1. The formation of the imidazolinones 8 from 7 and the NMR data of 8

<u>7</u>	Solvent (R <sub>4</sub> OH)	O <sub>2</sub> consumed (mmole)	CO <sub>2</sub> evolved (mmole)	Yield of <u>8</u> (%)	<u>8</u>	NMR data for protons of <u>8</u> <sup>a</sup>				
						>N-Me	-NH- <sup>b</sup>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
<u>7a</u>	MeOH	0.81	0.60	78	<u>8a</u>	7.18s 7.18s	4.10	7.00d (J=5)	6.80s	6.80s
<u>7c</u>	MeOH	1.23	1.10	28	<u>8a</u>					
<u>7a</u>	MeOH-d <sub>4</sub>	0.57	ND <sup>c</sup>	28	<u>8b</u>	7.18s	4.15	7.02d (J=5)	6.82s	—
<u>7a</u>	EtOH	0.80	ND	33	<u>8c</u>	7.18s	4.15	7.02d (J=5)	6.83s	8.73t (J=7) 6.38 qd (J=7, J=9) <sup>d</sup> 6.72qd (J=7, J=9) <sup>d</sup>
<u>7b</u>	MeOH	1.04	0.57	71	<u>8c</u>					
<u>7b</u>	MeOH-d <sub>4</sub>	0.58	ND	24	<u>8d</u>	7.20s	4.40	7.03d (J=5)	8.81t (J=7) 6.60m	—
<u>7b</u>	EtOH	0.71	0.73	25	<u>8e</u>	7.18s	4.20	7.05d (J=5)	8.82t (J=7) 6.60m	8.82t (J=7) 6.60m
<u>7d</u>	MeOH	0.76	1.20	57	<u>8f</u>	7.22s	4.35	8.75t (J=7) 6.58qd (J=7, J=5)	6.84s	6.84s
<u>7d</u>	MeOH-d <sub>4</sub>	0.84	ND	83	<u>8g</u>	7.20s	4.25	8.75t (J=7) 6.60qd (J=7, J=5)	6.84s	—

<sup>a</sup> Chemical shifts were given by  $\tau$ -value. Coupling constants (parentheses) were given by c.p.s.

The following abbreviations were used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

<sup>b</sup> These NH-protons appeared as a broad singlet.

<sup>c</sup> Not determined.

<sup>d</sup> This signal was analyzed by decoupling experiments.

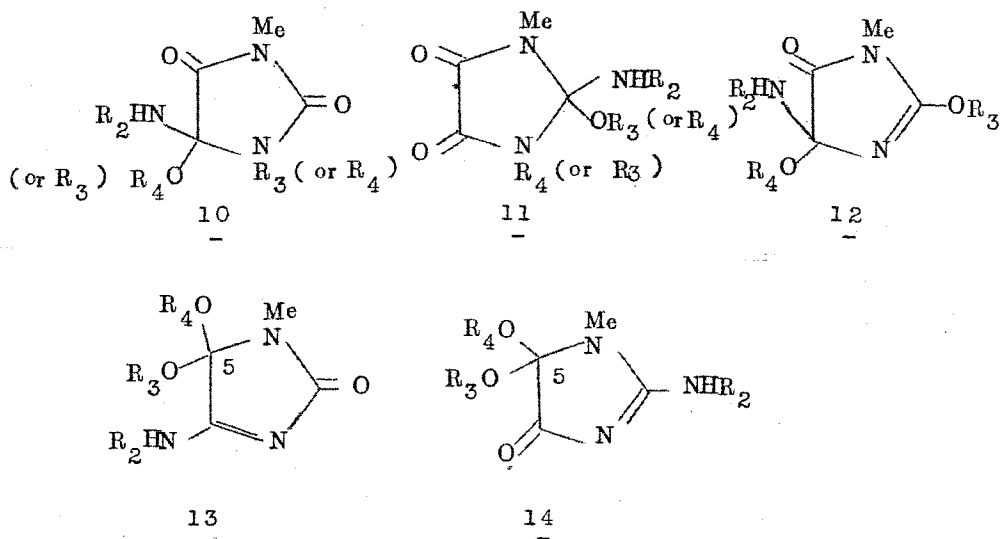
NMR spectra of these two products are quite analogous to those of the imidazolinones 8a, 8b, and 8c (Table 1). Furthermore, in the NMR spectrum of 8e signals attributed to the two ethoxy groups appear magnetically equivalent as those attributed to the two methoxy group of 8a.

Similarly, 1-ethyl-3,7-dimethyl-8-methoxyxanthine (7c) gave 8a, the same product as that from 7a in methanol, in 28% yield. The result clearly indicates that the 1-N-ethyl group was expelled in the course of the formation of 8a from 7c. In the case of the photosensitized oxygenation of 7a in ethanol, the fate of the 1-N-methyl group was found to be the formation of ethyl N-methylcarbamate (9;  $R_1=Me$ ,  $R_4=Et$ ) which was obtained in 68% yield.

In order to gain information on the origin of the N-methylamino group ( $-NH-R_2$  in formula 8) of the imidazolinone 8a, 8b, 8c, 8d, and 8e, the photooxygenation of 1,3-diethyl-7-methyl-8-methoxyxanthine (7d) was carried out. Thus 7d gave 8f (57%) in methanol and 8g (83%) in methanol- $d_4$ . The UV, IR, and NMR spectra of both products are quite analogous to those

of the imidazolinones obtained above, but the NMR spectra of 8f and 8g exhibit signals attributed to an -NH-Et group (Table 1). The results established that the 4-alkylamino group (-NH-R<sub>2</sub>) of the imidazolinones 3a-8g is derived from the 3-N-alkyl group of the starting xanthine derivatives 7a-7d.

Although the above results strongly support structure 8 for the imidazolinones, if we assume that drastic skeletal changes did occur in the course of the photooxygenation, other possible structures 10, 11, 12, 13, and 14 for the photooxidation product would be also taken into consideration. Among these, 10, 11, and 12 are ruled out from the fact that the photooxygenation of 7b in methanol afforded the same product 8c as the product obtained from 7a in ethanol.. For the formation of 13 and 14 from 7, one must consider complicated mechanisms involving migration of the alkoxy carbon atom at 8-position of 7 to 5-position of 13 and 14. In order to eliminate such unusual mechanisms, a tracer experiment using [8-<sup>14</sup>C] -labeled 3-methoxycaffeine (7a<sup>\*</sup>) was carried out.

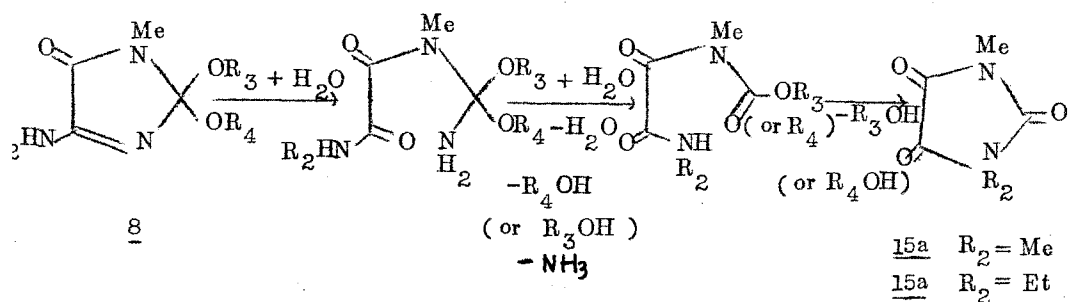


The imidazolinones 8 are extremely sensitive to hydrolysis. Thus, on treatment with aqueous acetic acid at room temperature 8a, 8c, and 8e yielded 1,3-dimethylparabanic acid (15a) in 91, 54, and 45% yield, respectively. On the other hand 8f was hydrolyzed under the same conditions to give no 1,3-dimethylparabanic acid (15a) but only 1-ethyl-3-methylparabanic acid (15b) (26%). The results can be explained by a mechanism shown in Scheme 1.

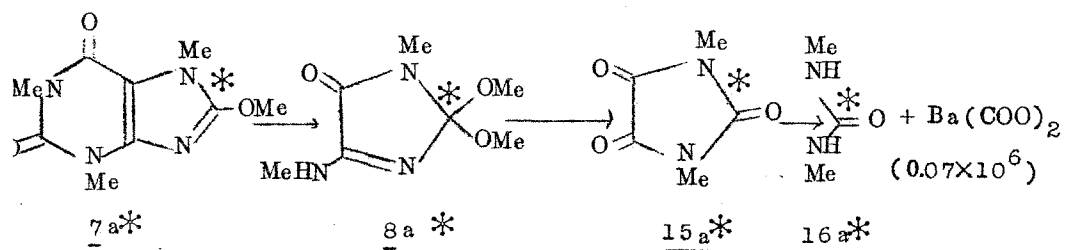
The derivation of 8a to 1,3-dimethylparabanic acid (15a) was applied to the tracer experiment. The (8-<sup>14</sup>C) -labeled 8-methoxycaffeine (7a<sup>\*</sup>) was prepared,

and it was submitted to photosensitized oxygenation under the standard conditions. The radioactive imidazolinone (8a\*) obtained was hydrolyzed with aqueous acetic acid to yield the active 1,3-dimethylparabanic acid (15a\*) which was then hydrolyzed with baryta into active 1,3-dimethylurea (16a\*) and practically inactive barium oxalate<sup>8</sup>. The data are shown in Scheme 2. The results clearly demonstrate that the 2-carbon atom of 8a was derived from the 8-carbon atom of 7a.

Scheme 1



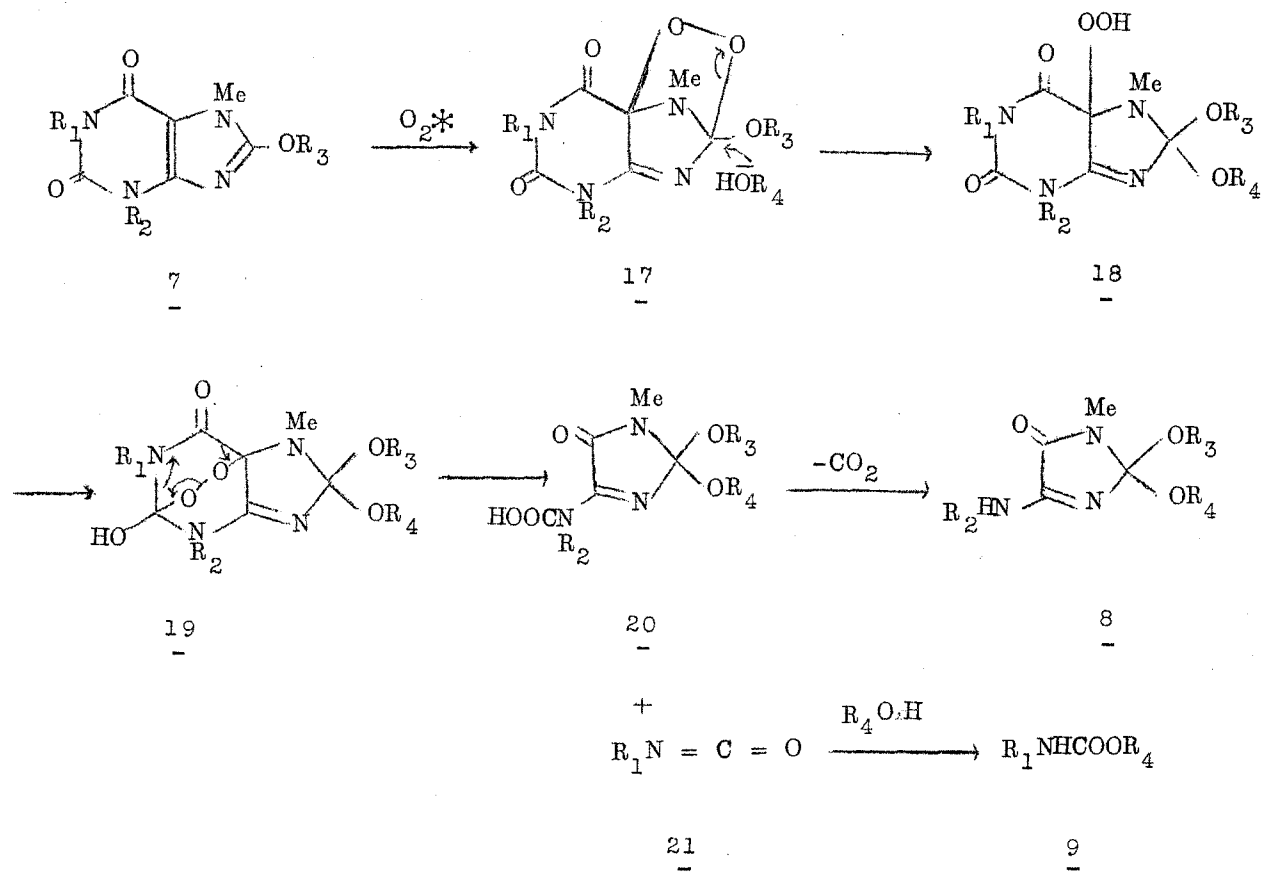
Scheme 2



$3.26 \times 10^6 \text{ cpm/mmole}$  ( $3.01 \times 10^6$ )

( $2.80 \times 10^6$ )( $2.70 \times 10^6$ )

The formation of the imidazolinones 8 from the trialkyl-8-alkoxy-xanthines 7 can be rationalized by scheme shown in Scheme 3. On the basis of the previous findings<sup>3</sup>, it is most probable that singlet oxygen attacks the starting material 7 to form a 5,8-endo-peroxide intermediate (17). Such a cyclic peroxide has been proposed for the initial intermediate in the photosensitized oxygenation of xanthine (1)<sup>2</sup>, 9-phenylxanthine,<sup>3</sup> and 1,3-dimethyl-9-phenylxanthine.<sup>3</sup> The solvent alcohol ( $R_4OH$ ) adds to the intermediate peroxide 17 to form an alkoxy hydroperoxide 18 which is, then, tautomerized to a six-membered cyclic peroxide 19. A similar mechanism involving such a tautomerization has been proposed for reactions involving a ketone hydroperoxide<sup>3,9,10</sup>. Peroxide 19 loses alkyl isocyanate (21) to give 20 which can be decarboxylated to form the imidazolinone 8. The alkyl isocyanate reacts with the solvent alcohol to give alkyl N-alkyl-carbamate (9) which was isolated from the reaction mixture in one case.



Scheme 3



### 5.3. Experimental

#### 5.3.1. Photosensitized Oxygenation of 8-Methoxycaffeine (7a).

##### A. In MeOH

A solution of 8-methoxycaffeine<sup>11</sup> (7a) (1.00 g, 4.46 mmoles) in MeOH-CHCl<sub>3</sub> (20:1, 100 ml) containing rose bengal (50 mg) was irradiated at room temperature by a 100 W high-pressure mercury lamp through a Pyrex cooling jacket. During the irradiation oxygen was bubbled by a circulating pump through a sintered-glass joint which was attached at the bottom of the reaction vessel. Oxygen consumption was manometrically followed. Carbon dioxide liberated was trapped with Ba(OH)<sub>2</sub> aq. Oxygen consumption was ceased after oxygen (90 ml, 3,6 mmoles) had taken up in 1 hr. Carbon dioxide was determined by weighing barium carbonate precipitated. After removal of the solvent in vacuo, the residue was dissolved in 20 ml of acetone-ether (1:3). The solution, when cooled at -70° with a dry ice-acetone bath, deposited crystals (0.65 g, 78%). Recrystallization from acetone gave 1-methyl-2, 2-dimethoxy-4-methylamino-3-imidazolin-5-

one (8a), m.p. 114-115°.  $\lambda_{\text{max}}^{\text{EtOH}}$  218 m $\mu$  ( $\epsilon$  28000),  
252 m $\mu$  ( $\epsilon$  6200),  $\nu_{\text{max}}^{\text{Nujol}}$  3350, 1750, and 1665  
cm<sup>-1</sup>. (Found: C, 44.91; H, 7.06; N, 22.49.

C<sub>7</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub> requires: C, 44.91; H, 7.06; N, 22.45%).

When the irradiation was made with a tungsten lamp, virtually same results were obtained. In the absence of rose bengal no oxygen consumption was observed and the starting material was recovered quantitatively.

#### B. In CD<sub>3</sub>OD

A solution of 7a (0.40 g, 1.8 mmoles) in CD<sub>3</sub>OD (9 ml) containing rose bengal (5 mg) was photooxidized under the same conditions. The mixture was worked up as described above to give 8b (90 mg, 28%), m.p. 114-115°.

#### C. In EtOH

A solution of 7a (4.00 g, 17.8 mmoles) in EtOH (100 ml) and CHCl<sub>3</sub> (20 ml) containing rose bengal (50 mg) was photooxidized in the standard manner for 3 hr. VPC analysis (silicon DC at 70°, with DMF as an internal standard) of the mixture revealed that the mixture contained ethyl N-methylcarbamate (9) (1.25 g, 68%). Fractional distillation of the mixture at the ordinary atmosphere gave pure ethyl N-methylcarbamate, b.p. 170°.

which was identical with an authentic sample (by IR and VPC). The residue was chromatographed on a neutral alumina column (60 g). Elution with  $\text{CHCl}_3$  (180 ml) gave 1,3-dimethylparabanic acid (15a) (0.12 g, 5%). Further elution with  $\text{CHCl}_3$  (300 ml) gave a semisolid (2.8 g). Recrystallization from acetone gave 1-methyl-2-ethoxy-2-methoxy-4-methylamino-3-imidazolin-5-one (8c) (1.20 g, 33%), m.p. 103-104°.  $\lambda_{\text{max}}^{\text{EtOH}}$  218 m $\mu$  ( $\epsilon$  27800), 252 m $\mu$  ( $\epsilon$  6200),  $\nu_{\text{max}}^{\text{Nujol}}$  3300, 1730, and 1665  $\text{cm}^{-1}$ . (Found: C, 47.63; H, 7.63; N, 20.59.  $\text{C}_8\text{H}_{15}\text{N}_3\text{O}_3$  requires: C, 47.75; H, 7.51; N, 20.88%).

### 5.3.2. Photosensitized Oxygenation of 8-Ethoxycaffeine (7b).

#### A. In MeOH

A solution of 8-ethoxycaffeine<sup>11</sup> (7b) (2.00 g, 8.4 mmoles) in  $\text{MeOH-CHCl}_3$  (20:1, 100 ml) containing rose bengal (50 mg) was photooxidized for 1 hr. The solvent was evaporated in vacuo and the residue was crystallized from acetone-ether (3:1, 20 ml). Recrystallization from acetone yielded crystals (1.20

g, 71%), m.p. 103-104°, which were identical with 8c obtained above (by mixture m.p., IR and NMR).

B. In CD<sub>3</sub>OD

A solution of 7b (0.30 g, 1.3 mmoles) in CD<sub>3</sub>OD (9 ml) containing rose bengal (10 mg) was photooxidized as usual. After treatment of the mixture as described above, 8d (60 mg, 24%) was obtained, m.p. 103-105°.

C. In EtOH

A solution of 7b (2.00 g, 8.35 mmoles) in EtOH (150 ml) and CHCl<sub>3</sub> (5 ml) containing rose bengal (20 mg) was photooxidized using tungsten lamp for 5 hr. After removal of the solvent in vacuo, the residue was chromatographed on a neutral alumina column (50 g). Elution with CHCl<sub>3</sub> (250 ml) gave a semisolid. Recrystallization from acetone yielded 8e (0.45 g, 25%), m.p. 108-109°.

$\lambda_{\text{max}}^{\text{EtOH}}$  218 m $\mu$  ( $\epsilon$  28000), 252 m $\mu$  ( $\epsilon$  6240),  $\nu_{\text{max}}^{\text{Nujol}}$  3300, 1715, and 1650 cm<sup>-1</sup>.

5.3.3. Photosensitized Oxygenation of 1-Ethyl-3,7-dimethyl-8-methoxyxanthine (7c).

A solution of 1-ethyl-3,7-dimethyl-8-methoxyxanthine<sup>12</sup> (7c) (0.70 g, 2.9 mmoles) in MeOH (80 ml) and CHCl<sub>3</sub> (5 ml) containing rose bengal (20 mg) was photo-

oxidized in the standard manner for 3 hr. After removal of the solvent, the residue was chromatographed on a neutral alumina column (15 g). Elution with  $\text{CHCl}_3$  (200 ml) gave crystals (0.15 g, 28%), m.p. 113-114 $^\circ$ , which were identical with 8a (by mixture m.p. IR, and NMR).

#### 5.3.4. 1,3-Diethyl-7-methyl-8-methoxyxanthine (7d)

This compound was prepared from 1,3-diethylxanthine<sup>13</sup> by the known method for the synthesis of 8-alkoxycaffeine<sup>11</sup>, m.p. 143-147 $^\circ$ , NMR ( $\text{CDCl}_3$ ),  $\tau$  8.70 (triplet ( $J = 6.5$  cps), 3H,  $> \text{N-CH}_2\text{-Me}$ ), 8.67 (triplet ( $J = 6.5$  cps), 3H,  $> \text{N-CH}_2\text{-Me}$ ), 6.34 (singlet, 3H,  $> \text{N-Me}$ ), 5.90 (singlet, 3H,  $-\text{OMe}$ ), 5.90-6.20 (multiplet, 4H,  $> \text{N-CH}_2\text{-Me}$ ). (Found: C, 52.06; H, 6.40; N, 22.29.  $\text{C}_{11}\text{H}_{16}\text{N}_4\text{O}_3$  requires: C, 52.37; H, 6.39; N, 22.21%).

#### 5.3.5. Photosensitized Oxygenation of 1,3-diethyl-7-methyl-8-methoxyxanthine (7d).

##### A. In MeOH

A solution of 7d (1.00 g, 4.0 mmoles) in MeOH (50 ml) and  $\text{CHCl}_3$  (5 ml) containing rose bengal

(20 mg) was photooxidized under the standard conditions for 2 hr. After removal of the solvent in vacuo, the residue was chromatographed on a neutral alumina column (20 g). Elution with 50 ml of benzene- $\text{CHCl}_3$  (1:1) yielded an oil (0.45 g, 57%), which crystallized upon standing overnight. Attempts to recrystallization were unsuccessful. In order to obtain an analytical sample, the crystals were again chromatographed on an alumina column to give pure 8f, m.p.  $75-77^\circ$ .  $\lambda_{\text{max}}^{\text{EtOH}}$  218  $\text{m}\mu$  (26000), 253  $\text{m}\mu$  ( $\epsilon$  6000),  $\nu_{\text{max}}^{\text{Nujol}}$  3300, 1725, and 1655  $\text{cm}^{-1}$ . (Found: C, 47.43; H, 7.49; N, 20.51.  $\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_3$  requires: C, 47.75; H, 7.51; N, 20.88%).

#### B. In $\text{CD}_3\text{OD}$

A solution of 7d (0.50 g, 2 mmoles) in  $\text{CD}_3\text{OD}$  (18 ml) containing rose bengal (10 mg) was photooxidized under the standard conditions. The solvent was removed in vacuo and the residue was chromatographed on a neutral alumina column (10 g). Benzene (50 ml) eluted 8g (0.34 g, 83%), which crystallized upon standing overnight, m.p.  $74-75^\circ$ .

#### 5.3.6. Hydrolysis of 8 with Aqueous Acetic Acid.

A solution of 8a (40 mg) in H<sub>2</sub>O containing three drops of acetic acid was kept at room temp for 24 hr. To the solution H<sub>2</sub>O (50 ml) and CHCl<sub>3</sub> (50 ml) was added. The chloroform layer was separated and evaporated to dryness. Crystallization of the residue from acetone gave, 1,3-dimethylparabanic acid (15a) (28 mg, 91%). Similarly, 8c and 8e was hydrolysed with aqueous acetic acid to give 1,3-dimethylparabanic acid (15a) in 54 and 45% yield, respectively, but no 1-ethyl-3-methylparabanic acid could be detected on TLC. On similar treatment with aqueous acetic acid, 8f (50 mg) afforded 1-ethyl-3-methylparabanic acid (15b) (10 mg, 26%). Recrystallization from acetone gave crystals, m.p. 45-48° (lit.<sup>14</sup> m.p. 44°). No 1,3-dimethylparabanic acid was detected by TLC analysis of the mother liquor.

5.3.7. (8-<sup>14</sup>C)-8-Methoxycaffeine (7a\*).

Theophylline labeled at 8-position with [<sup>14</sup>C] was prepared from 1,3-dimethyl-4,5-diaminouracil<sup>13</sup> and formic acid containing 0.5 mc of [<sup>14</sup>C]-formic acid according to the method of Speer et al<sup>13</sup>.

Methylation of  $[8-^{14}\text{C}]$ -theophylline with dimethyl sulfate gave  $[8-^{14}\text{C}]$ -caffeine which, on chlorination and subsequent methoxylation according to the method of Huston<sup>11</sup>, gave  $[8-^{14}\text{C}]$ -8-methoxycaffeine (7a\*) ( $3.26 \times 10^6$  cpm/mmole).

5.3.8. Photosensitized Oxygenation of  $[8-^{14}\text{C}]$ -8-Methoxycaffeine (7a\*) in Methanol.

A solution of  $[8-^{14}\text{C}]$ -8-methoxycaffeine (7a\*) (4.00 g, 16.8 mmole,  $3.26 \times 10^6$  cpm/mmole) in MeOH- $\text{CHCl}_3$  (6:1, 200 ml) containing rose bengal (50 mg) was photooxidized under the standard conditions. Carbon dioxide liberated was trapped as barium carbonate (1.70 g, 48%, 660 cpm/mmole). Recrystallization of the product gave  $[2-^{14}\text{C}]$ -8a (2.32 g, 69%,  $3.01 \times 10^6$  cpm/mmole), m.p. 113-114°.

5.3.9. Acid Hydrolysis of  $[2-^{14}\text{C}]$ -1-Methyl-2,2-dimethoxy-4-methylamino-3-imidazolin-5-one(8a\*)

A solution of 8a\* (2.00 g, 10.7 mmole) in aqueous acetic acid was treated as described above to give  $[2-^{14}\text{C}]$ -1,3-dimethylparabanic acid (15a\*) (1.05 g, 69%,  $2.804 \times 10^6$  cpm/mmole). A solution of 15a\* (0.95 g,



6.7 mmoles) in 3% aqueous barium hydroxide (100 ml) was kept at 40-50° for 15 min according to the procedure of Behrends et al<sup>8</sup>. Barium oxalate (1.45 g, 95%,  $7.05 \times 10^4$  cpm/mmmole) precipitated was collected by filtration. The filtrate was evaporated in vacuo to dryness. The residue was extracted with acetone (50 ml). After removal of the solvent, the residue was crystallized benzene to give  $\left[^{14}\text{C}\right]$ -N,N-dimethylurea (0.17 g, 29%,  $2.698 \times 10^6$  cpm/mmmole).

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## 6. Photosensitized Oxygenation of 9-Phenylguanine

### 6.1. Introduction

A large number of workers demonstrated that irradiation of bacteria or virus by visible light in the presence of a sensitizing dye causes inactivation<sup>1</sup>. This photodynamic inactivation is known to be mainly due to the selective degradation of guanine residues in DNA by photosensitized oxygenation<sup>2</sup>. It is, therefore, of interest to understand the chemical mechanisms of the photodynamic degradation of guanine derivatives.

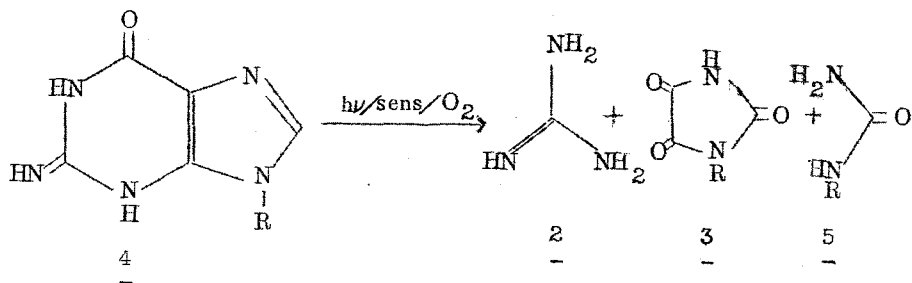
It has been demonstrated that lumichrome-sensitized oxygenation of guanine(1) in an aqueous solution results in a complex mixture of products from which guanidine(2), parabanic acid (3a), and carbon dioxide can be detected<sup>3</sup>. On the other hand, Sastry and Gordon reported that the photosensitized oxygenation of guanosine(4a) affords guanidine (2), ribosylurea (5a), urea (6) and ribose (7)<sup>4</sup>. Since

the chemical mechanisms of these reactions remains unknown, the investigation on the photosensitized oxygenation of 9-phenylguanine (4b), a simple analogue of guanosine (4a), has been carried out. Furthermore, an attempt to propose possible mechanisms for the photosensitized oxidation of guanine (1) and guanosine (4a) is presented in this section.

## 6.2. Results and Discussion

When an alkaline aqueous solution of 9-phenylguanine(4b) was submitted to photosensitized oxygenation in the presence of rose bengal, 1.4 moles of oxygen was consumed. Acidification of the reaction mixture liberated carbon dioxide (0.6 mole), which was detected by converting into barium carbonate. The TLC analysis of the acidified mixture showed that it consisted of a complex mixture of products, among which phenylurea (5b) and a trace of N-phenylparabanic acid (3b) was detected. From the reaction mixture guanidine-hydrochloride (2) could be obtained in 5% yield as an only isolable product. The above reaction is quite similar

to that of guanosine (4a)<sup>4</sup>.



From the above result and a series of the results mentioned in the previous sections of Chapter I on the photosensitized oxygenation of various purine derivatives<sup>5,6</sup>, it is proposed possible mechanisms for these reactions.

The structure of the peroxide intermediate in the photosensitized oxygenation of purines depends upon the structural feature of their imidazole moiety. When two double bonds are present in the imidazole moiety as in 8, an endo-peroxide such as 9 might be the intermediate. Thus, the formation of allantoin (10) from xanthine (8) is well rationalized by a mechanism involving a 5,8-endo-peroxide (9) as shown in Scheme 1<sup>6</sup>. Similarly, 4,5-dimethoxyuric

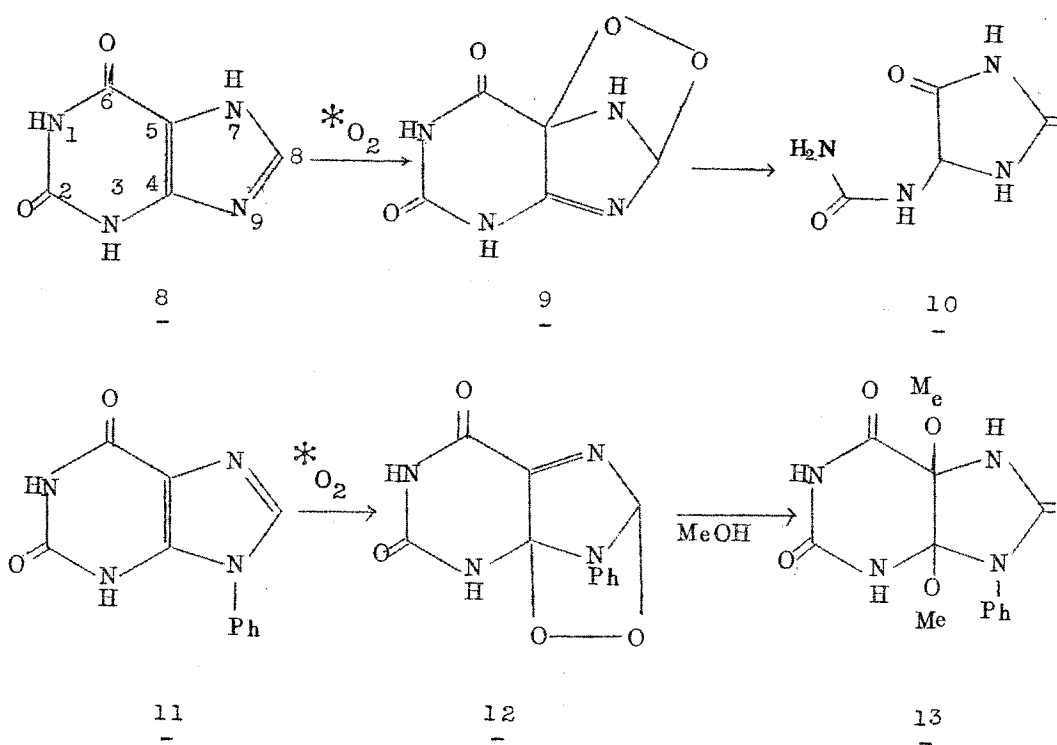
acid derivative\* (13) is considered to be formed via a 4,8-endo-peroxide (12) from 9-phenylxanthine (11)<sup>7</sup>. The results suggest that the intermediate in the photosensitized oxygenation of guanine (1) may be a 5,8-endo-peroxide (14), which is converted to a hydroperoxide 15 as shown in Scheme 2. On the other hand, in the photosensitized oxygenation of 9-phenylguanine (4b) or guanosine (4a) a 4,8-endo-peroxide (16) could be the intermediate, which is similarly converted to 15 (Scheme 2). The hydroperoxide 15 is then dehydrated to give 17.

As the hydrolytic decomposition modes of 17, two pathways may be considered. Via path a, 17 is hydrolysed to give 18, which is then decomposed to yield N-formylguanidine<sup>8</sup>(19) and N-alkylparabanic acid (3). Oxidative decarboxylation of 19 might afford guanidine (2) and carbon dioxide. The above mechanism can also account for the Behrends' results<sup>9,10</sup> with <sup>14</sup>C-labeled guanine, namely parabanic acid (3), guanidine (2), and carbon dioxide contain C-8, C-2, and C-6 carbon atoms of the original guanine, respectively, and guanidine (2) and carbon dioxide arise from the same

intermediate.

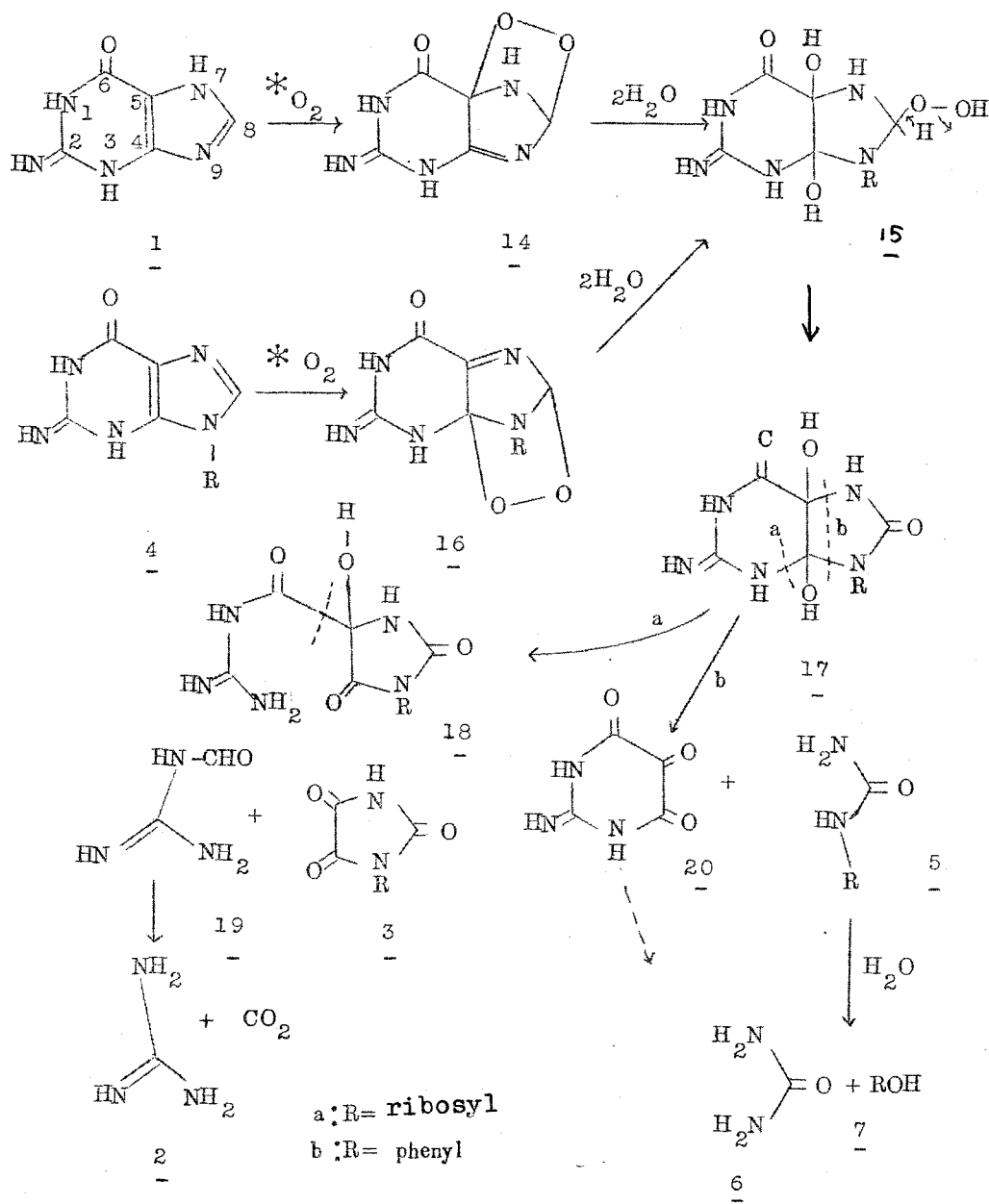
The intermediate 16 may be hydrolysed via an alternative route (path b) to give 2-iminoalloxan (20) and N-alkylurea (5). 2-Iminoalloxan is extremely unstable under the conditions employed. Paper chromatographic analysis showed that the reaction mixture of 9-phenylguanine (4b) contained one of the decomposition products of 20. The result suggests that 20 may be also involved as an intermediate in the photosensitized oxygenation of 9-phenylguanine.

Although mechanisms involving an energy-<sup>10</sup> or electron-transfer<sup>11</sup> between guanine (1) and the excited triplet sensitizer have been postulated in the photosensitized oxygenation of guanine, it is more favorable from the results previously mentioned in Chapter I, that the singlet oxygen is involved in the present photosensitized oxygenation<sup>5</sup>. However, the detailed mechanism for the initial attack of an oxygen molecule to purine derivatives remains to be elucidated.



Scheme 1





Scheme 2

### 6.3. Experimental

#### 6.3.1. Materials

9-Phenylguanine (4b) was prepared according to the method of Robins et al<sup>12</sup>: Phenylparabanic acid (3b) was prepared by the condensation of phenylurea (5b) with ethyl oxalate according to the method of Dieckmann et al<sup>13</sup>. 2-Iminoalloxan (20) was prepared by the method of Kaess et al<sup>14</sup>.

#### 6.3.2. Photosensitized Oxygenation of 9-Phenylguanine (4b).

A solution (pH 13.1) of 9-phenylguanine (4b) (400 mg, 2 mmoles) in 0.07 N sodium hydroxide (180 ml, 15 mmoles) containing rose bengal (20 mg) was irradiated by a 100 W high pressure mercury lamp for 2 hr. After the oxygen (62 ml, 2.8 mmoles) had been consumed, the reaction mixture (pH 12.2) was acidified to pH 2.0 with 6 N hydrochloric acid. Carbon dioxide (1.2 mmoles) liberated was trapped with an aqueous solution of barium hydroxide. The acidified mixture was concentrated in vacuo to dryness. The residue was dissolved in hot methanol (100 ml). The methanol extract was

subjected to thin layer and paper chromatography. The thin layer chromatographic analysis (silica gel G,  $\text{CHCl}_3$ -EtOH, (10:3)) of the methanol extract showed that it consisted of at least eight compounds, among which phenylurea (5b) at Rf 0.50 and a trace of phenylparabanic acid (3b) at Rf 0.25 were detected.

The paper chromatogram of the extract was compared with that of the methanol extract which was obtained in the same treatment of an alkaline aqueous solution (pH 13.2) of 2-iminoalloxan (20). The reaction mixture from 2-iminoalloxan (20) showed four spots (unchanged starting material (20) at Rf 0.35 and three compounds at Rf 0.30, 0.10, and the origin) on paper chromatogram using n-butanol-acetic acid-water (4:1:5) as a solvent. The compound at Rf 0.35 was also detected by the paper chromatographic analysis of the photooxidation mixture of 4b.

After concentration of the methanol extracts from 9-phenylguanine, the residue was chromatographed on a neutral alumina column (20 g). Elution with 500 ml of ethyl acetate-EtOH (1:1) gave guanidine-

hydrochloride (2) (11 mg, 5%), which was identified by the comparison of its IR spectrum with that of an authentic sample. Further elution with EtOH gave a brown oily materials (250 mg).

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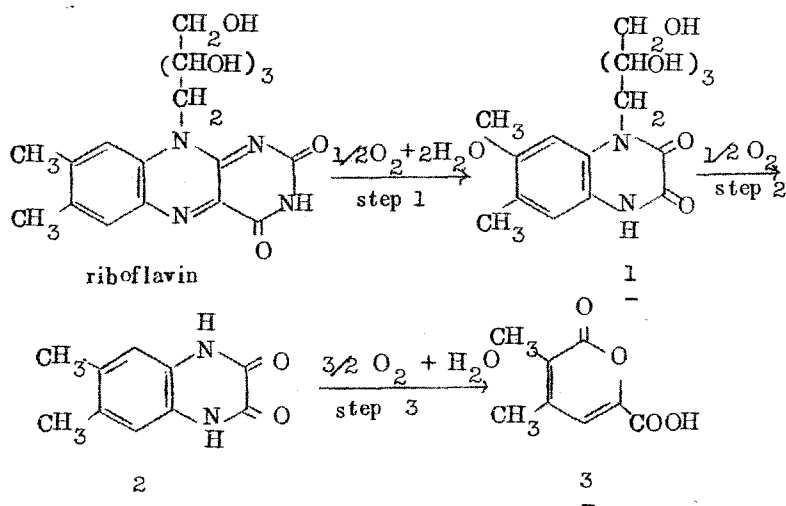
CHAPTER II  
OXIDATION OF QUINOXALINE-2,3-DIOLS  
AS A POSSIBLE MODEL FOR THE BIOLOGICAL  
DECOMPOSITION OF RIBOFLAVIN

1. Introduction

The first significant observation on the biological decomposition of riboflavin was reported by Foster et al<sup>1</sup>, who demonstrated that Pseudomonas riboflavinus catalyzed the hydrolysis of riboflavin to lumichrome and ribitol, followed by the oxidation of the latter to carbon dioxide. Miles and Stadtman<sup>2</sup> reported that a microorganism degrades riboflavin to 6,7-dimethyl-9-(2'-hydroxyethyl) isoalloxazine under anaerobic conditions. Recently the isolation of 6,7-dimethyl-9-(2'-carboxyethyl) isoalloxazine, related to the above isoalloxazine, from urine of sheeps was reported.<sup>3</sup> Such types of the degradation of the side chain of riboflavin seem to be related to the photochemical degradation of riboflavin<sup>4</sup>.

Another type of the biological decomposition of riboflavin has been investigated by Stadtman and collabo-

rators<sup>5,6,7,8,9</sup>. They demonstrated that a different strain of P. riboflavinus degrades the isoalloxazine portion of the molecule. Thus riboflavin is oxidized to 3,4-dimethyl-6-carboxy- $\alpha$ -pyrone (3) via 2,3-diketo-quinoxaline derivatives, 1, and 2.



Although the mechanism of this microbial degradation of riboflavin has not yet been established, it has been suggested that the metabolic conversion of 1 to 2 may involve a mixed function oxygenase enzyme and that the conversion of 2 ~~to~~ <sup>to</sup> 3 is a multistep process in which the presence of molecular

oxygen plus any one of several co~~2~~substrates capable of pyridine nucleotide linked oxidation are required<sup>9</sup>. In order to contribute to the elucidation of the mechanism of this biological oxidation, investigation on the oxidation of the metabolic intermediates, 1 and 2, and also of a simple analog of 2, quinoxaline-2, 3-diol (4), were examined by chemical means and presented in this chapter. To our best knowledge, the chemical oxidation of quinoxaline-2,3-diol derivatives has not appeared in the literature, although quinoxaline itself undergoes oxidative cleavage of its benzene ring under drastic conditions to give pyrazine-2,3-dicarboxylic acid<sup>10</sup>.



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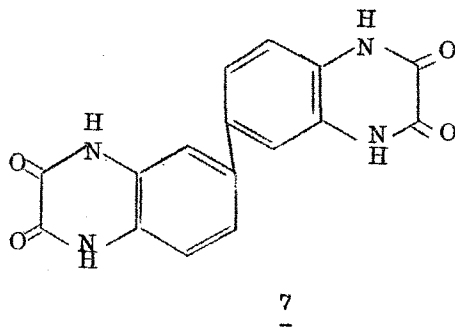
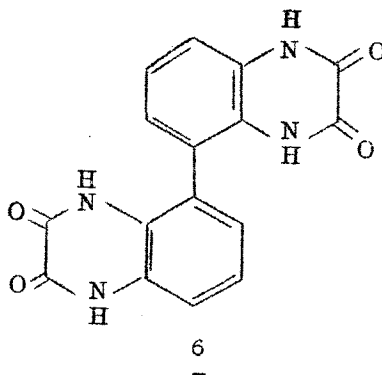
## 2. Alkaline Ferricyanide Oxidation of Quinoxaline-2,3-diols

### 2.1. Results

The autoxidation of three quinoxaline-2,3-diol derivatives, i.e., 1-ribity-2,3-diketo-6,7-dimethyl-1, 2,3,4-tetrahydroquinoxaline (1), 6,7-dimethylquinoxaline-2,3-diol (2), and quinoxaline-2,3-diol (4), was examined in alkaline media. These compounds were quite stable under the conditions employed. However, alkaline ferricyanide which is known to be a one-electron transfer oxidizing agent was found to be capable of oxidizing these quinoxalinediols. Quinoxaline-2,3-diol (4) was slowly oxidized with alkaline ferricyanide in a nitrogen atmosphere to give two products. One was obtained in 27% yield and identified as cis, trans-muconic acid (5) through its spectral data and through its conversion to trans, trans-muconic acid by ultraviolet irradiation. The other product, which was soluble in aqueous alkali but insoluble in various organic solvents, was obtained in low yield and could not be isolated in pure form.

Its UV spectrum (Figure 1) is similar to that of the

starting material except for an additional band at 265  $m\mu$  which is usually present in biphenyl derivatives<sup>1</sup>. This suggests that the structure of the second product may be represented either by a 5,5' (6) or by a 7,7' dimer (7). Since the 5,5' dimer (6) which was obtained by an unambiguous synthesis, was not identical with the product, we tentatively assigned structure 7 to the second product.



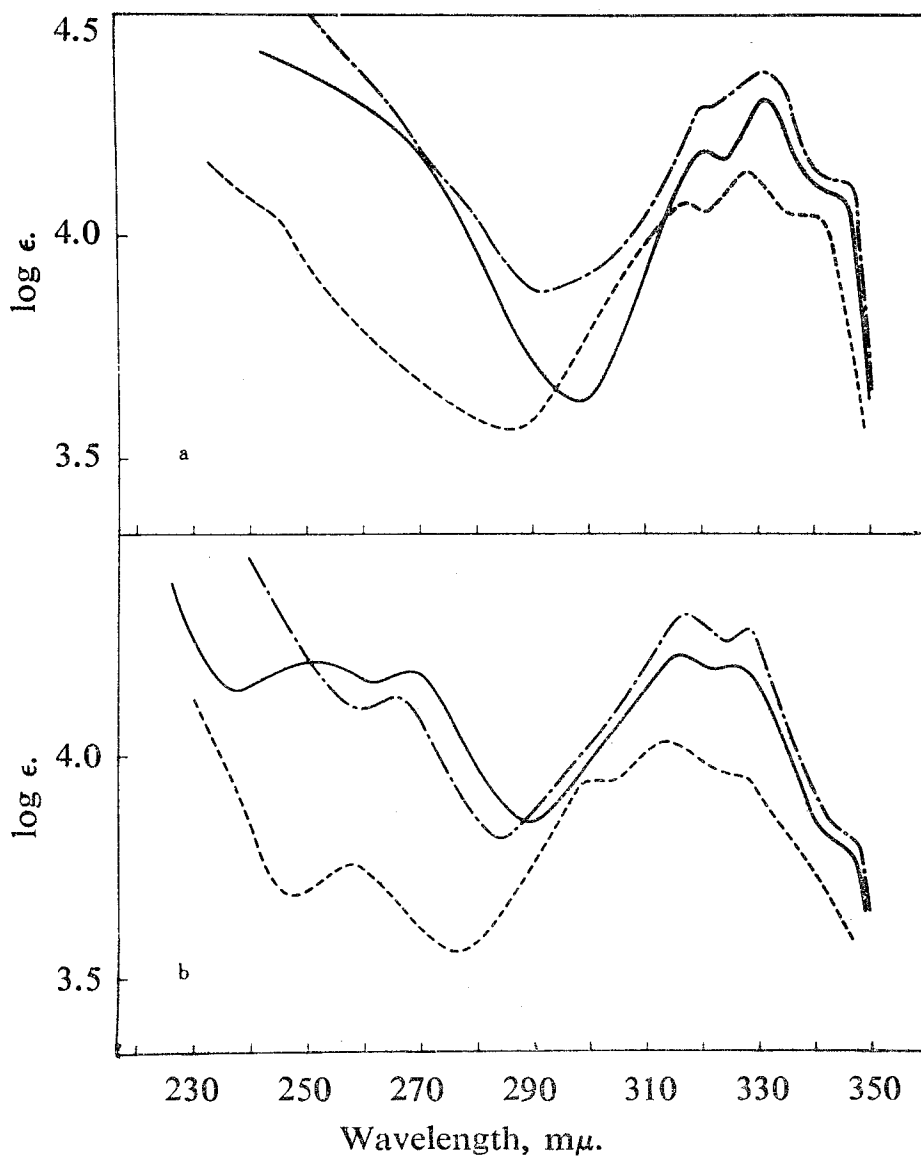
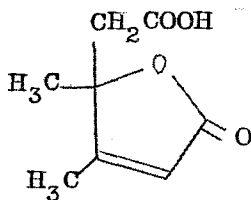


FIGURE 1. Ultraviolet Absorption Spectra of Quinoxaline-2,3-diol (4) (----), (7,7'-biquinoxaline)-2,2',3,3'-tetrol (7) (-----), and (5,5'-biquinoxaline)-2,2',3,3'-tetrol (6) (--- - ---). (a) In 0.1 N sodium hydroxide. (b) In 0.1 N hydrochloric acid.

Oxidation of 6,7-dimethylquinoxaline-2,3-diol (2) with alkaline ferricyanide was easier than that of 4 and resulted in the formation of a lactonic acid  $C_8H_{10}O_4$  (16 %), an acid  $C_{10}H_8N_2O_4$  (20 %), and polymers. Structure 8 was assigned to the lactonic acid on the basis of its spectral properties. The IR ( $\nu_{\text{max}}^{\text{KBr}}$  1740, 1720, and 1640  $\text{cm}^{-1}$ ) and UV ( $\lambda_{\text{max}}^{\text{EtOH}}$  212  $\text{m}\mu$ ) absorption spectra showed an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone ring and a carboxylic group. In the NMR spectrum, signals of two methyl groups were observed; one is attributed to the 4-methyl group ( $\tau$  8.45, singlet) and the other to the 3-methyl group ( $\tau$  7.90, doublet,  $J = 1.5$  cps). Signals of one vinyl proton ( $\tau$  4.18, quartet,  $J = 1.5$  cps) and two carboxymethyl protons ( $\tau$  7.17, AB quartet,  $J_{AB} = 18.5$  cps) were also observed.



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The spectral properties of the acid  $C_{10}H_8N_2O_4$  led to its assignment as 6-carboxy-7-methylquinoxaline-2,3-diol (9). The UV spectrum showed similar absorptions to that of 2 (Figure 2) and the IR spectrum showed bands of a carboxylic group at  $1690\text{ cm}^{-1}$ , a carbonyl group at  $1675\text{ cm}^{-1}$ , and an amide group at  $3500\text{ cm}^{-1}$ . The NMR spectrum showed the presence of two aromatic protons ( $\tau$  2.89 and 2.63) and a methyl group ( $\tau$  7.55, singlet) in the molecule. These spectral data are in agreement with structure 9.

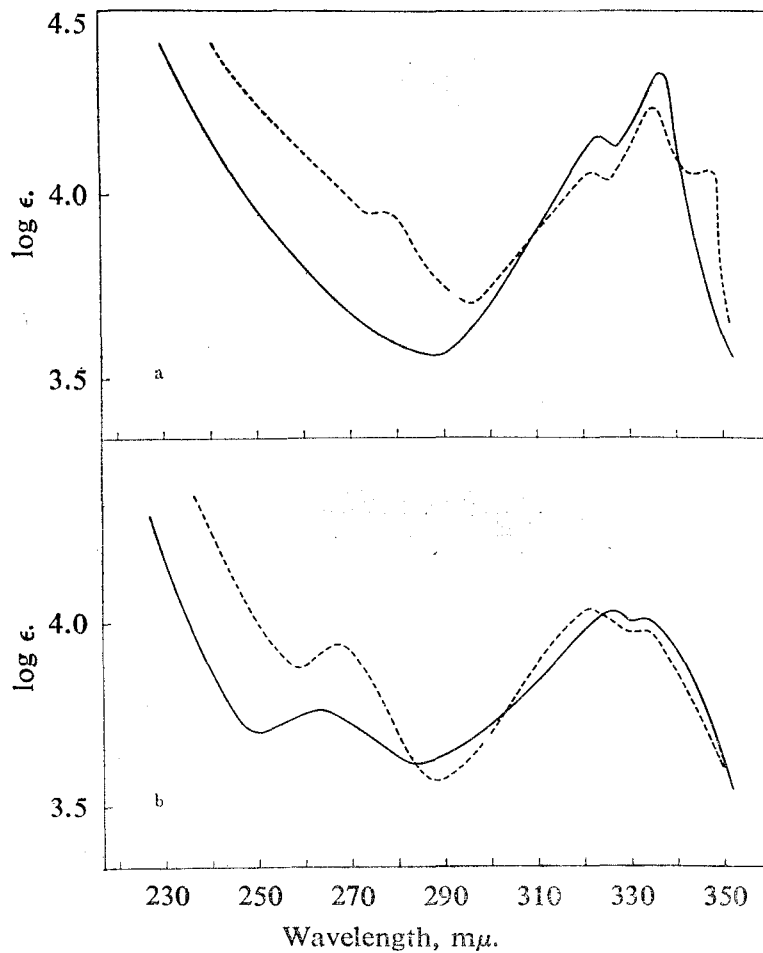
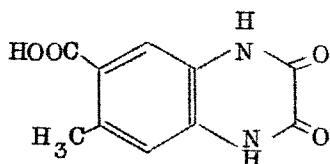


FIGURE 2: Ultraviolet absorption spectra of 6,7-dimethylquinoxaline 2,3-diol (2) ( — ) and 6-methyl-7-carboxyquinoxaline-2,3-diol (9) ( - - - ). (a) In 0.1 N sodium hydroxide. (b) In 0.1 N hydrochloric acid.



9

Oxidation of 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (1) with ferricyanide was carried out in 0.1 N aqueous alkaline solution, since 1 is known to be more labile to alkali than 2, yet stable in 0.1 N alkali. Thin layer chromatography showed that the reaction product consisted of at least eight compounds. Of these products three 2, 8, and 9, were isolated. Some of the starting material was also detected. The results indicate that in the course of the ferricyanide oxidation 1 is converted to 2 by oxidative elimination of the ribityl side chain.

## 2.2. Experimental

### 2.2.1. Materials

Quinoxaline-2,3-diol (4) was prepared according to the method described by Newbald and Spring<sup>2</sup>;  $\lambda_{\text{max}}^{0.1 \text{ NaOH}}$



315 ( $\epsilon$  12,000), 326 (14,500), 340 m $\mu$  (11,000).

6,7-Dimethylquinoxaline-2,3-diol (2) was prepared according to the method of Tsai et al.<sup>3</sup>;  $\lambda_{\text{max}}^{0.1 \text{ N NaOH}}$

321 ( $\epsilon$  13,000), 335 (17,500), and 345 m $\mu$  (12,000).

1-Ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (1) was prepared according to a modification of the procedure of Miles et al.<sup>4</sup>. A mixture of 5 g of 2-(N-ribitylamino)-4,5-dimethylaniline and 100 g of diethyl oxalate was heated at 140-150° under nitrogen for 2 hr. Removal of diethyl oxalate followed by trituration of the residual oil with 100 ml of methanol yielded a grayish green solid. Two crystallizations from acetic acid afforded 1.5 g of 1 as colorless needles: mp 257-260°;  $\lambda_{\text{max}}^{0.1 \text{ N NaOH}}$  322 ( $\epsilon$  12,000), 334 (13,500), and 350 m $\mu$  (9500). (Found: C, 55.16; H, 6.35; N, 8.85.  $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_6$  requires: C, 55.55; H, 6.22; N, 8.64 %).

#### 2.2.2. Alkaline Ferricyanide Oxidation of Quinoxaline-2,3-diol (4).

To a solution of 5.0 g (0.03 mole) of quinoxaline-2,3-diol in 500 ml of 2 N sodium hydroxide

was added 30 g (0.09 mole) of potassium ferricyanide. The mixture was stirred under nitrogen for 24 hr. Acidification of the mixture with 12 N hydrochloric acid deposited unreacted starting material (3.0 g) which was collected by filtration. The filtrate was extracted with ether, and a yellow solid (0.48 g, 27% on the basis of the reacted 4), obtained after evaporating the extract, was recrystallized from ethyl acetate to give cis, trans-muconic acid: mp 188-190°;  $\lambda_{\text{max}}^{\text{EtOH}}$  255 m $\mu$  ( $\epsilon$  24,500) and  $\nu_{\text{max}}^{\text{Nujol}}$  900 and 720 cm<sup>-1</sup>. (Found: C, 50.49; H, 4.59. C<sub>6</sub>H<sub>6</sub>O<sub>4</sub> requires: c, 50.71; H, 4.26%).

Ultraviolet irradiation of this compound in the presence of iodine<sup>5</sup> yielded trans,-trans-muconic acid whose infrared spectrum was identical with that of an authentic sample.

The aqueous layer from the ether extraction was further extracted with phenol. After evaporation of the phenol, the residue showed two fluorescent spots ( $R_f$  0.58 and 0.40) on a paper chromatogram using 1-butanol saturated with 2 N aqueous ammonia. The substance corresponding to the spot of  $R_f$  0.58 was identified as the starting material 4. The residue was

adsorbed on cellulose power and eluted with 1-butanol saturated with 2 N aqueous ammonia. The eluate, which showed the spot of  $R_f$  0.40 on a paper chromatogram, was neutralized with acetic acid and evaporated to dryness. The residual solid obtained was dissolved in 8 ml of water and the solution was acidified with 2 N hydrochloric acid to yield a solid (45 mg) which did not melt below  $300^\circ$ :

$\lambda_{\text{max}}^{0.1 \text{ N HCl}}$  265, 300, 314, and 328 m $\mu$ .

2.2.3. (5,5'-Biquinoxaline) 2,2',3,3'-tetrol (6).

2,2', 3,3'-Tetraaminobiphenyl (60 mg), which was synthesized according to the method of Dieteren and Konigsberger<sup>1</sup>, was suspended in 25 ml of diethyl oxalate. The mixture was heated at  $120-130^\circ$  under nitrogen for 3 hr. The resulting solid was dissolved in 3 ml of 0.5 N sodium hydroxide and acidified with 2 N hydrochloric acid to yield 38 mg of 6, which did not melt below  $300^\circ$ ;  $\nu_{\text{max}}^{\text{Nujol}}$  ca.  $1700 \text{ cm}^{-1}$  (br); NMR (dimethyl sulfoxide)  $\tau$  3.2-2.7 (six aromatic protons, multiplet), -0.8 (two NH protons, singlet), and -2.05 (two NH protons singlet). (Found: C,

59.71; H, 2.98; N, 17.98.  $C_{16}H_{10}N_4O_4$  requires: C, 59.66; H, 3.13; N, 17.39 %).

2.2.4. Alkaline Ferricyanide Oxidation of 6,7-Dimethyl quinoxaline-2,3-diol (2).

To a solution of 3.0 g (0.015 mole) of 6,7-dimethyl-quinoxaline-2,3-diol in 400 ml of 2 N sodium hydroxide was added 40 g (0.12 mole) of potassium ferricyanide and the mixture was stirred under nitrogen for 10 hr. The reaction mixture was adjusted to pH 2 with 2 N hydrochloric acid and extracted with 1-butanol. The extract was chromatographed on a silica gel column. Elution with chloroform-acetone (5:1) yielded a crystalline solid (0.41 g, 16%). Recrystallization from ethyl acetate gave the lactonic acid 8 as crystals: mp 97-98°; NMR ( $CDCl_3$ )  $\tau$  8.45 (three protons, singlet), 8.90 (three protons, doublet,  $J = 1.5$  cps), 7.17 (two protons, AB quartet,  $J_{AB} = 18.5$  cps), and 4.18 (1 proton, quartet,  $J = 1.5$  cps);  $\nu_{max}^{KBr}$  1740, 1720, and 1640  $cm^{-1}$ ;  $\lambda_{max}^{EtOH}$  212  $m\mu$  ( $\epsilon$  15,700). (Found: C, 56.76; H, 5.72.  $C_8H_{10}O_4$  requires: C, 56.46; H, 5.92%).

Further elution with acetone yielded a crystalline solid (0.70 g, 20%). Recrystallization from acetone-

methanol gave 6-methyl-7-carboxyquinoxaline-2,3-diol (9) as crystals, which did not melt below 300°.

This compound was soluble in aqueous sodium bicarbonate;  $\lambda_{\text{max}}^{0.1 \text{ N HCl}}$  265 ( $\epsilon$  5900), 308 (6200), 330 (10,300), and 332 m $\mu$  (9500);  $\nu_{\text{max}}^{\text{Nujol}}$  1690 and 1675 cm<sup>-1</sup>; NMR (D<sub>2</sub>O-NaOD)  $\tau$  7.55 (three protons, singlet), 2.89 (one proton, singlet), and 2.63 (one proton, singlet). (Found: C, 54.03; H, 3.23; N, 12.25. C<sub>10</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub> requires: C, 54.55; H, 3.06; N, 12.72%).

Further elution with ethanol yielded polymers (0.8 g).

#### 2.2.5. Alkaline Ferricyanide Oxidation of 1-Ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (1).

A solution of 0.50 g (1.5 mmoles) of 1 and 5 g (1.5 mmoles) of potassium ferricyanide in 100 ml of 0.1 N sodium hydroxide was stirred under nitrogen for 5 hr. The mixture was acidified with 2 N hydrochloric acid and extracted with 1-butanol. Paper chromatography of the extract showed four major and two minor spots in short-wave ultraviolet light. Thin layer chromatography on silica gel with benzene-

ethyl formate-formic acid (5:2:1) showed at least eight spots. Of these products three were isolated by chromatography on cellulose powder with 1-butanol saturated with 2 N ammonia and identified by comparison with authentic samples as 6,7-dimethylquinoxaline-2,3-diol (2)(IR), the lactonic acid 8 (thin layer chromatography), and the acid 9(UV and paper chromatography). Some starting material 1 was also detected among the products. Other products were not obtained in a pure form.

### 3. Photosensitized Oxygenation of Quinoxaline-2,3-diols

#### 2.1. Results

In the photooxidation of quinoxaline 2,3-diol (4) in the presence or absence of rose bengal as a sensitizer no oxygen consumption was observed and the starting material (4) was recovered quantitatively. Photosensitized oxidation of 6,7-dimethylquinoxaline -2,3-diol (2) in 2.0 N aqueous alkaline solution in the presence of rose bengal gave at least nine products, which were detected on a thin layer chromatogram, after 4 moles of oxygen had been consumed. In the absence of rose bengal the starting material (2) was recovered quantitatively (Table I).

Under similar conditions and in the presence of rose bengal 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (1) consumed 5 mole equiv of oxygen. When irradiation was interrupted after the consumption of 0.5 mole equiv of oxygen, paper chromatographic analysis (Figure 3) of the reaction mixture showed that it consisted of 2,

the unchanged starting material 1, and an unidentified compound. After the consumption of 2 mole equiv of oxygen, 2 was isolated in 18% yield from the reaction mixture. When 1 was photooxidized until oxygen consumption ceased (5 mole equiv), 2 was no more found in the reaction mixture which was shown by thin layer chromatography to consist of at least seven products (Figure 3). These results demonstrate that one of the initial steps of the photosensitized oxidation of 1 is the oxidative deribitylation of 1 to 2.



TABLE I: Photosensitized Oxidation of Quinoxaline Derivatives 1, 2, and 4.

Substance	Solvent(N)	Reaction Time (hr)	Sensitizer <sup>a</sup>	Oxygen consumed
<u>4</u>	NaOH(2)	10	RB	None
<u>2</u>	Pyridine	8	RB	None
<u>2</u>	NaOH(2)	15	None	None
<u>2</u>	NaOH(2)	18	RB	4 mole equiv
<u>2</u> <sup>b</sup>	NaOH(2)	25	RB	4 mole equiv
<u>1</u>	NaOH(0.5)	10	RB	5 mole equiv

<sup>a</sup>Rose bengal (RB) was used as the sensitizer. <sup>b</sup>An aqueous copper sulfate solution (ca. 10%) was used as a filter.

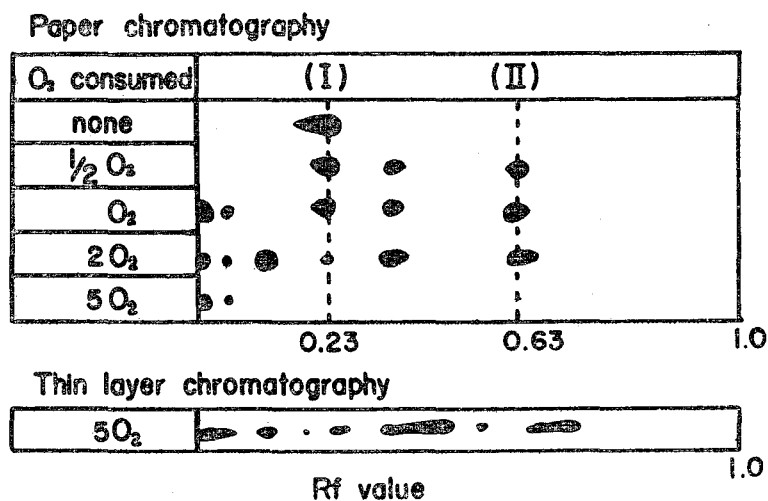


FIGURE 3: Chromatography of the photosensitized oxidation products of 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (1). Paper chromatography: 1-butanol saturated with 2 N ammonia. Thin layer chromatography: benzene-ethyl formate-formic acid (5:2:1).

## 2.2. Experimental

### 2.2.1. Photosensitized Oxidation of 6,7-Dimethylquinoxaline-2,3-diol (2).

In a typical run, a solution of 1.0 g (5.2 mmoles) of 6,7-dimethylquinoxaline-2,3-diol (2) and 20 mg of rose bengal in 100 ml of 2 N sodium hydroxide was irradiated at room temperature with a 100-w high-pressure lamp (Ushio Type UM 100) having a Pyrex cooling jacket. During the irradiation oxygen was bubbled through a sintered-glass joint which was attached at the bottom of the reaction vessel. Oxygen consumption was followed manometrically. After 450 ml(20 mmoles) of oxygen had been consumed, the reaction mixture was acidified with hydrochloric acid and extracted with 1-butanol. The extract showed at least nine spots on thin layer chromatogram (silica gel, benzene-ethyl formate-formic acid (5:2:1). The products were isolated in impure form after chromatography on a silica gel column. Other results obtained under various conditions are summarized in Table I.

3.2.2. Photosensitized Oxidation of 1-Ribityl-2,3-diketo  
6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (1).

A solution of 1.2 g (3.8 mmoles) of 1 and 20 mg of rose bengal in 200 ml of 0.5 N sodium hydroxide was treated as described above. The irradiation was interrupted when 175 ml (8 mmoles) of oxygen had been consumed. One-half (100 ml) of the reaction mixture was acidified and extracted with 1-butanol. The butanol extract gave six fluorescent spots on a paper chromatogram (Figure 3). The major spots were identified as 1 and 2. From the butanol extract, 80 mg of crystals was isolated by preparative paper chromatography (solvent, 2 N  $\text{NH}_4\text{OH}$ -1 butanol). The product was identified as 2 by comparison of its infrared spectrum with that of an authentic sample.

The other half of the mixture was further irradiated until a total of 10 mmoles of oxygen/3.8 mmoles of 1 had been consumed. The mixture was treated as described above. The butanol extract showed no more 1 and 2 on a paper chromatogram. On a thin layer chromatogram at least seven spots were detected (Figure 3). These products were not further investigated.

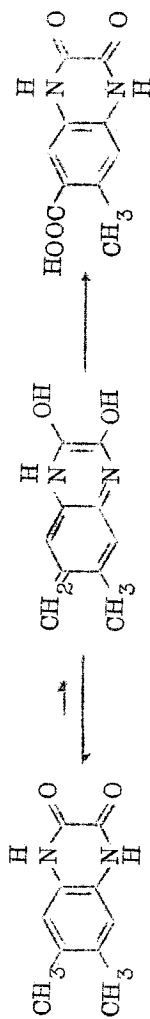
#### 4. Discussion

Alkaline ferricyanide oxidation and photosensitized oxygenation, which were chosen as moderate oxidizing methods for the quinoxaline-2,3-diols 1, 2, and 4, appear to be closely related to some biological oxidation. Alkaline ferricyanide is a one-electron transfer oxidizing agent. One-electron transfer oxidations are known to occur in the biosynthesis of various natural products such as alkaloids<sup>6</sup>.

Photosensitized oxygenation of various biological systems, such as proteins and nucleic acids, is known as photodynamic action<sup>7,8</sup>. Photosensitized oxygenation also seems to be closely related to oxygenases and to some oxidases. For example, it has been reported that tyrosine is photooxidized in the presence of a sensitizer to give 3,4-dihydroxyphenylalanine<sup>9</sup>, representing a model for the enzymatic hydroxylation of tyrosine<sup>10</sup> and of 3,4-dimethylphenol<sup>11</sup> by phenolases. Tryptophan is also photooxidized under similar conditions to give various

degradation products<sup>12</sup>, including kynurenine and 3-hydroxykynurenine, which are known metabolites of tryptophan formed under the influence of tryptophan pyrrolase<sup>13</sup>.

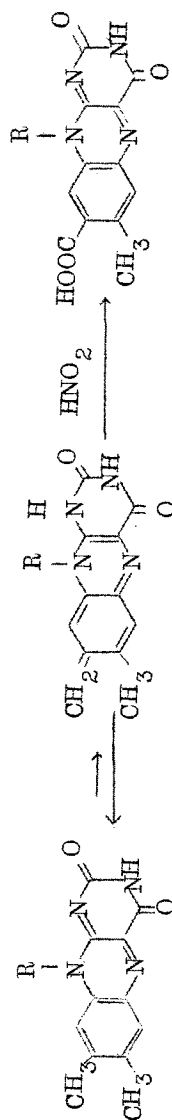
Whereas the reaction of quinoxaline-2,3-diol (4) itself with alkaline ferricyanide is relatively slow, 6,7-dimethylquinoxaline-2,3-diol (2) is easily oxidized by ferricyanide. Such a difference between the reactivities of 2 and 4 was also observed in the case of the photosensitized oxidation. This is probably due to hyperconjugation (as formula 10) involving one of the methyl groups at the 6,7 positions of 2. A similar hyperconjugation has been proposed for riboflavin (11, R = ribityl) by Hemmerich et al.<sup>14</sup>. The formation of 6-carboxy-7-methylquinoxaline-2,3-diol (9) in the ferricyanide oxidation of 2 is quite analogous to the oxidation of lumiflavin with nitrous acid, leading to 7, 10-dimethylisoalloxazine-8-carboxylic acid<sup>14</sup> (12).



2

10

9

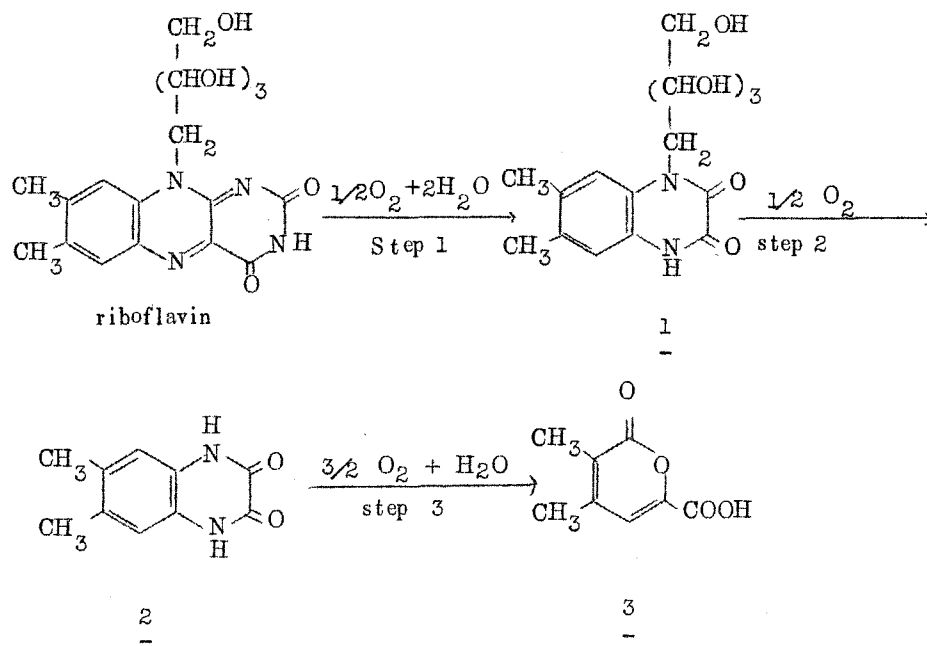


11

12

R = H : Lumiflavin  
 R = Ribityl : Riboflavin

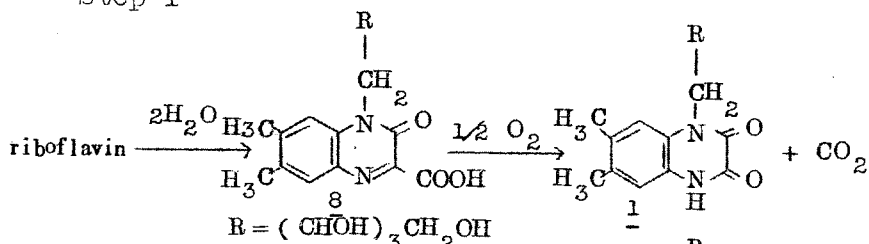
Scheme 1



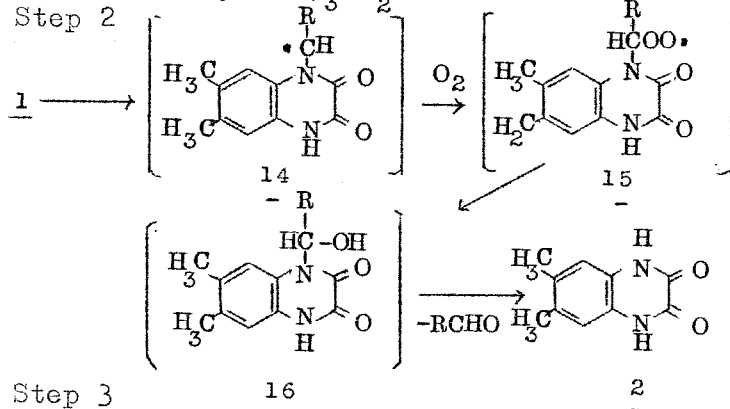


Scheme 2

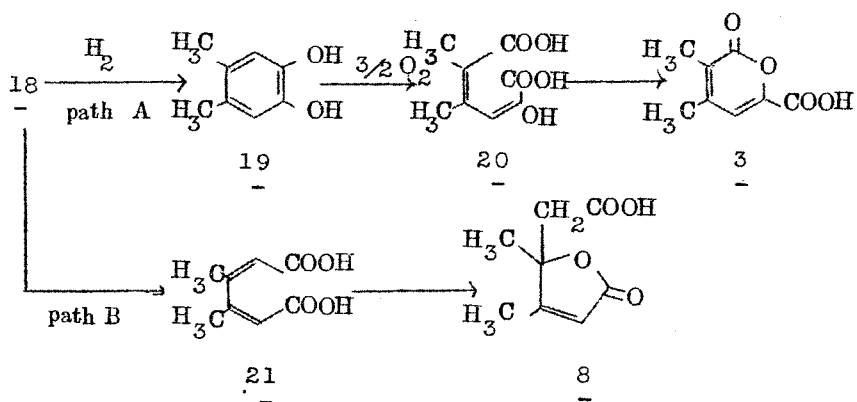
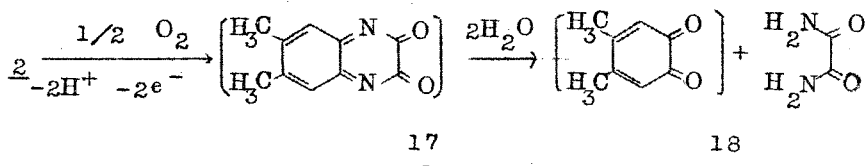
Step 1

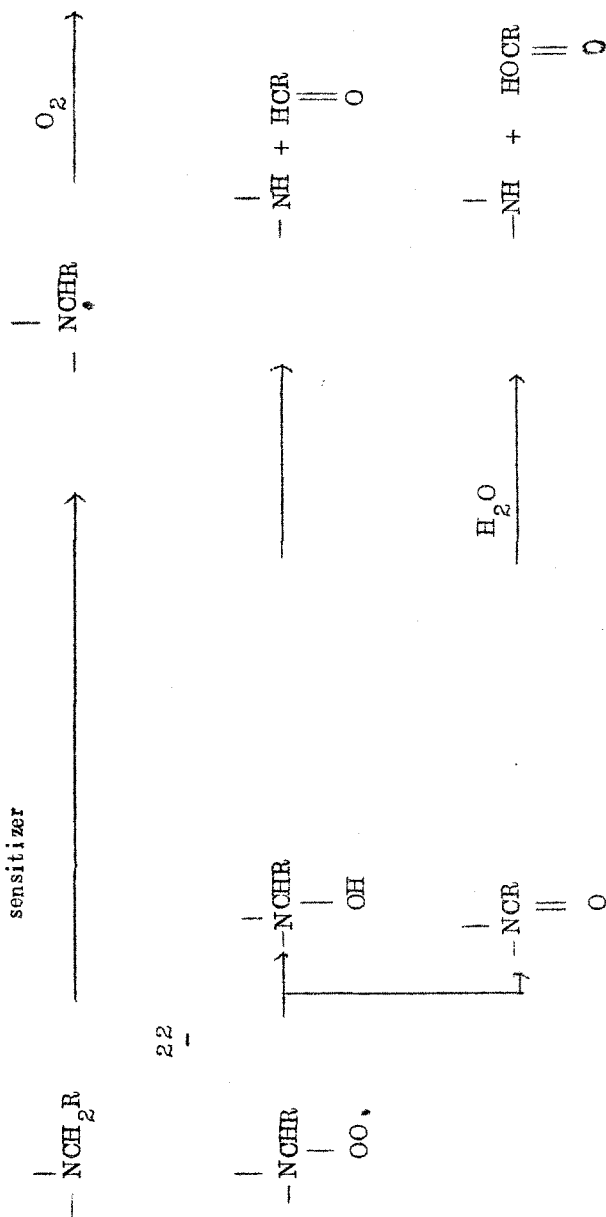


Step 2



Step 3



Photoexcited  
sensitizer

As shown in Scheme I, the bacterial degradation of riboflavin leading to 3,4-dimethyl-6-carboxy- $\alpha$ -pyrone (3) involves the following three steps: (1) conversion of riboflavin to 1, (2) deribitylation of 1 to 2, and (3) degradation of 2 and 3. A model reaction for step 1 has already been reported by Miles et al.<sup>15</sup>, who demonstrated that the alkaline hydrolysis of riboflavin followed by oxidation of the product 13 with peracetic acid yielded 1, as shown in Scheme 2. This result is consistent with the formulation by Stadtman and his group (Scheme 1) which requires two molecules of water and one atom of oxygen in step 1.

The formation of 2 from 1, either by ferricyanide oxidation or by photosensitized oxidation, represents a model for step 2. In connection with the mechanism of the deribitylation of 1, it should be mentioned that there are a few reports on the oxidative dealkylation of tertiary amines. Perline<sup>16</sup> reported that tertiary N-methylamines such as 1,2,6-trimethylpiperidine are demethylated by the action of potassium ferricyanide, although the mechanism is

not known. Furthermore, the photosensitized oxidation of certain amines or amides, which possess a partial structure of 22, results in the removal of the N-alkyl group or in the formation of acylamines, as shown in Scheme 3.

In the present model reactions for step 2, in particular in the photosensitized oxidation of 1, a similar mechanism, which involves radical intermediates 14 and 15, is applicable. Recently extensive mechanistic investigations on riboflavin photochemistry have been carried out by Moore and Metzler<sup>20,21</sup>. They suggested that the photochemical deribitylation of riboflavin might be initiated by an internal hydrogen abstraction from a CH on the ribityl chain by the photoexcited isoalloxazine group. Such a mechanism might not be applicable to the deribitylation of 1 in the photosensitized oxidation, because 1 is recovered unchanged on photooxidation without sensitizer. Since an energy transfer from the excited sensitizer to the substrate, which would cause excitation of the carbonyl group, appears unfavorable, it is concluded that an excited molecule of 1 is not involved in the present reaction. The radical

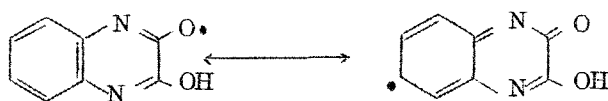
15 may be converted into 16 which is then cleaved to form 2. Since 2 and ribose have been found to be products in the enzymatic coribitylation<sup>3</sup> of 1, it is reasonable to assume that the enzymatic reaction also proceeds by an analogous mechanism.

For step 3, we suggest common intermediates (17 and 18) in both the enzymatic and chemical degradation of 2. Ferricyanide can abstract two electrons from the dianion of the quinoxalinediol (2) to form 17, which in turn is hydrolyzed to 4,5-dimethyl- $\alpha$ -benzoquinone (18) and oxamide. Further oxidative cleavage of 18 with ferricyanide appears to proceed via path A, which is different from the enzymatic cleavage via path B. The 2,3-bond fission of catechols frequently occurs in biological systems involving metapyrocatechase enzymes<sup>22</sup>. Thus, it is reasonable to assume that, in the course of the bacterial decomposition of riboflavin, 4,5-dimethylcatechol (19) which might be enzymatically derived from the quinone 18, is cleaved to 3,4-dimethyl-6-carboxy- $\alpha$ -pyrone (3) via the acid 20.

In contrast to such an enzymatic cleavage of

catechols, the chemical oxidation of catechols or o-quinones usually results in a 1,2-bond fission. For instance, in an alkaline ferricyanide oxidation 4,5-di-t-butylpyrogallol is cleaved at the bond between two hydroxy groups at the 3 and 4 positions to yield 3,6-di-t-butyl-6-carboxy-  $\alpha$ -pyrone<sup>23</sup>. Therefore, it can be rationalized that the o-quinone 18 is cleaved by alkaline ferricyanide to form  $\beta$ ,  $\beta$ -dimethyl-cis, cis-muconic acid (21) which is then cyclized by an intramolecular Michael-type addition to the lactonic acid 8.

The formation of cis, trans-muconic acid (5) in the alkaline ferricyanide oxidation of quinoxaline-2,3-diol (4) may also be rationalized by a similar mechanism. The formation of the dimer 7 in this oxidation can be explained by the coupling of a free radical (23) which is produced by the removal of one electron from 4.



23

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## CHAPTER III

### OXIDATION OF LIPOIC ACID AND ITS MODEL REACTION FOR THE ENZYMATIC ACYL-TRANSFER REACTION

#### 1. Introduction

In bacteria and animal cells lipoic acid is an important prosthetic group of multienzyme systems which generate acetyl CoA and succinyl CoA from pyruvate and  $\alpha$ -ketoglutarate, respectively. The enzyme bound lipoic acid undergoes a cycle of reductive acylation, acyltransfer and reoxidation. Although there has been numerous investigations<sup>1-4</sup> on the natural function of lipoic acid in  $\alpha$ -keto acid dehydrogenation complexes of various microorganisms, no significant observation on the metabolism of lipoic acid has been demonstrated. Furthermore, the function of  $\beta$ -lipoic acid, a natural occurring oxidation product of lipoic acid, in  $\alpha$ -keto acid dehydrogenase system remains unknown. In connection with the metabolism of lipoic acid, some oxidations of

lipoic acid were examined and presented in this Chapter. In order to suggest the possibility that  $\beta$ -lipoic acid could undergoes a acyl-transfer reaction in the enzyme systems, a chemical model reaction of  $\beta$ -lipoic acid with acetic anhydride is also described in this chapter.

#### References

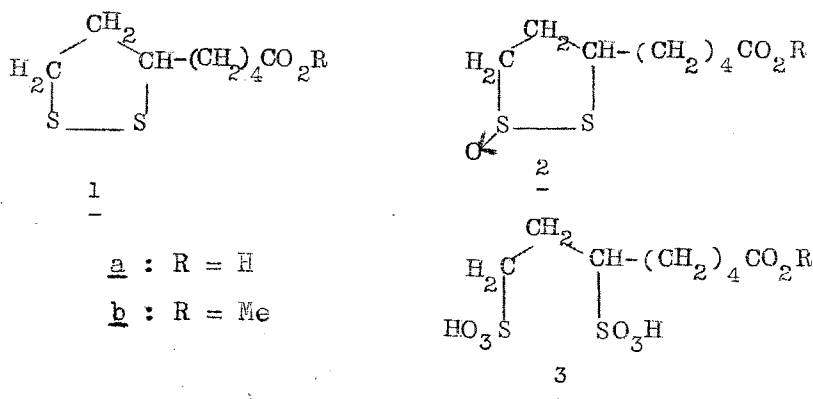
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## 2. Oxidation of Lipoic Acid

### 2.1. Introduction

Recent studies on the metabolism of  $\alpha$ -lipoic acid (1a) have demonstrated that  $^{35}\text{S}$ -labeled lipoic acid injected to animals gave in the urine about 10 radioactive substances including unmetabolized  $\alpha$ -lipoic acid and its sulfoxide form,  $\beta$ -lipoic acid (2a)<sup>1,2,3</sup>. Though the structure of these metabolites have not been elucidated yet, the greater part of them are supposed to be formed by oxidative degradation of the disulfide linkage of lipoic acid. As for the oxidation product of lipoic acid, only 6,8-disulfooctanoic acid (3a)<sup>4</sup> has been ascertained in addition to  $\beta$ -lipoic acid (2a). In the case of biotin, it has been known that biotin d-sulfoxide and biotin l-sulfoxide are naturally occurring oxidation products having a low biotin activity<sup>5,6</sup>, and biotin sulfone acts as an antagonist against certain microorganisms<sup>7</sup>. In order to contribute to the elucidation of the structure of the biological oxidative degradation products of lipoic acid,

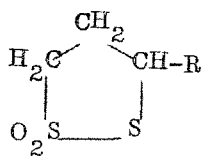
some oxidation reactions including photosensitized oxygenation of lipoic acid were investigated. In the photosensitized oxygenation of 1b mechanisms for the formation of 2b were briefly discussed. Furthermore, the microbiological and pyruvate oxidation activity of the oxidation products were also examined.



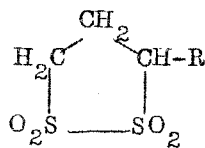
## 2.2. Results and Discussion

Peroxide oxidation and photosensitized oxygenation, both of which appear to be closely related to some biological oxidation, were chosen as moderate oxidizing methods for the  $\alpha$ -lipoic acid (1). Photosensitized oxygenation seems to be closely related to oxygenases and to some oxidases<sup>8</sup>. In the photosensitized oxygenation methyl  $\alpha$ -lipoate (1b) afforded methyl  $\beta$ -lipoate

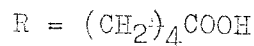
(2b). On the other hand, t-butylhydroperoxide oxidized la to give 2a and 8-sulfoneliipoic acid (4), and peracetic acid oxidation of la gave 6,8-disulfoneliipoic acid (5).



4



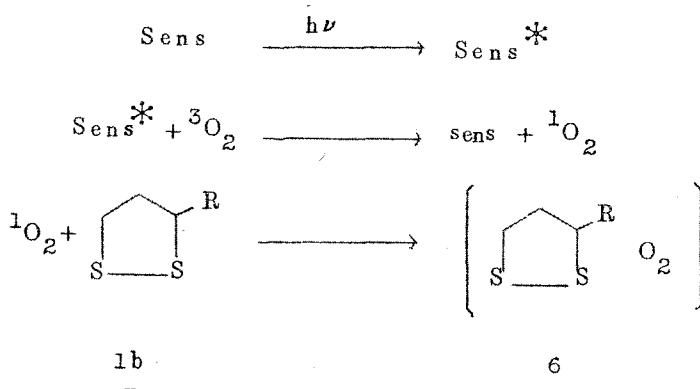
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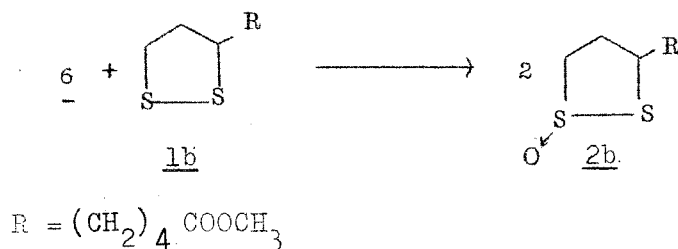


When a methanol solution of lb was irradiated by a tungsten lamp in the presence of rose bengal as a sensitizer under bubbling oxygen, 0.52 equivalent of oxygen was consumed. Thin layer chromatographic analysis of the reaction mixture showed that it consisted of methyl  $\beta$ -lipoate (2b) and polymeric products. No other product was detected on the thin layer chromatogram of the mixture. Methyl  $\beta$ -lipoate (2b) was isolated from the reaction mixture in 89% yield. In the absence of the sensitizer lb showed no oxygen consumption and was recovered unchanged under the condition. The results indicate that the excited state, possibly a triplet

state<sup>9</sup>, of the sensitizer may be involved in the present reaction. On the other hand, methyl  $\beta$ -lipoate (2b) was not further oxidized under the conditions employed.

The formation of 2b from 1b may be rationalized by a pathway shown in Scheme 1. The attack of an excited singlet oxygen molecule<sup>9</sup> to 1b gives an oxygen adduct 6, which then reacts with another molecule of 1b yielding 2b. The fact that 0.5 molar equivalent of oxygen is required in the formation of one mole of 2b, is compatible with the above mechanism. Such types of photosensitized oxygenation are also demonstrated in the cases of diemthyl sulfoxide<sup>10</sup> and triphenyl phosphine<sup>11</sup>. However, the structure of the oxygen adduct still remains to be elucidated.





Scheme 1

When  $\alpha$ -lipoic acid (1a) was oxidized with t-butylhydroperoxide in chloroform, paper chromatographic analysis (Fig. 1) of the reaction mixture showed that it consisted of  $\beta$ -lipoic acid (2a)(80%) at Rf0.35, the unreacted starting material (1a)(3%) at Rf0.60, and an unidentified compound (5%) at Rf 0.90. The unknown compound exhibited growth inhibitory effect against Streptococcus faecalis 10Cl. Radioactivity resulted from  $^{35}\text{S}$ -labeled  $\alpha$ -lipoic acid was also detected on the spot.

# Radiochromatogram

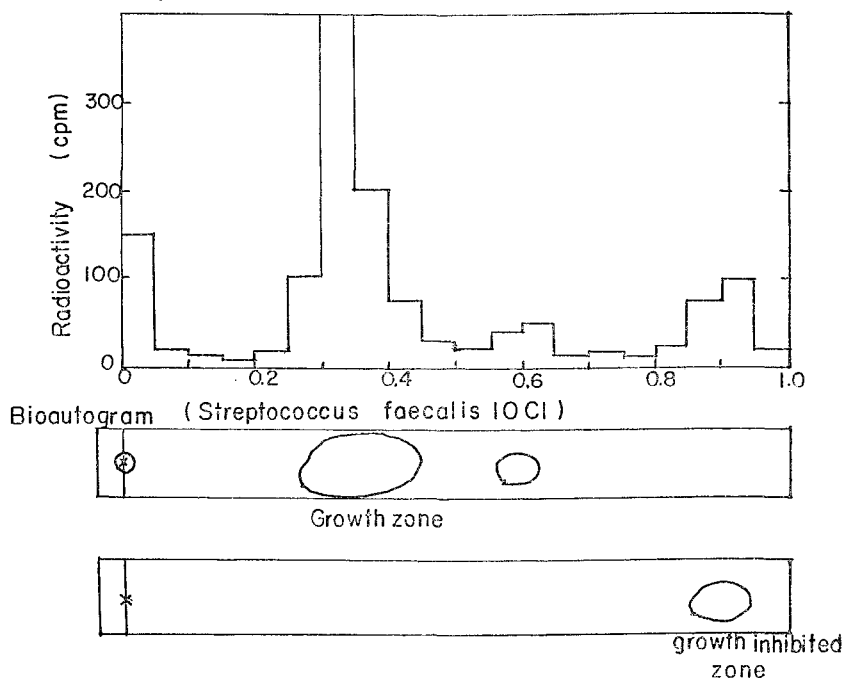
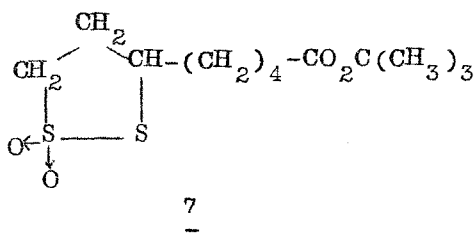


Fig. 1 Paper Chromatogram of an Oxidation Mixture of  $\alpha$ -Lipoic Acid with t-Butylhydroperoxide

The UV spectrum of the compound did not show the peak at 248 m $\mu$  due to a sulfoxide group. Its IR spectrum



exhibited characteristic peaks assignable to a sulfone group at  $1310\text{ cm}^{-1}(\nu_{\text{as}} \text{SO}_2)$  and  $1140\text{ cm}^{-1}(\nu_{\text{s}} \text{SO}_2)$ . The absorption bands attributed to an ester group were also observed. The IR spectrum of the compound was quite similar to that of t-butyl ester of 1a except to bands of a sulfone group. Taking into consideration of a steric hinderance of a bulky side chain, structure 7 was given to the compound.



8-Sulfonellipoic acid (4) was obtained by hydrolysis of 7. On paper chromatography, thin layer chromatography and paper electrophoresis, 4 exhibited behaviours analogous to those of  $\beta$ -lipoic acid (2a). Table 1 gives data for anti-lipoic acid action of 4. When 100 times as much 8-sulfonellipoic acid (4) was added to a medium containing lipoic acid, the growth of Streptococcus faecalis 10C1 was

markedly decreased.

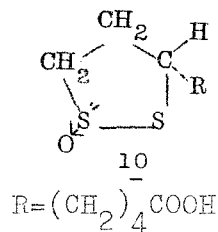
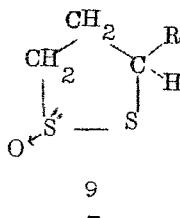
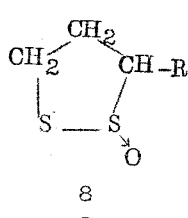
TABLE 1

Growth Inhibitory Effect of 8-Sulfoneliipoic  
acid Against Streptococcus faecalis 10C1

$\alpha$ -Lipoic acid	8-Sulfoneliipoic acid(4)	Bacterial growth
m $\mu$ g/ml	m $\mu$ g/ml	O.D.
1	0 0	0.225
1	10	0.320
1	100	0.135
1	300	0.136

When  $\alpha$ -lipoic acid (1a) was oxidized with 2 molar ratio of hydrogen peroxide in aqueous acetone, an unidentified compound (3%) was produced in addition to  $\beta$ -lipoic acid (2a) and the unreacted  $\alpha$ -lipoic acid (1a). The compound has the same molecular formula as 2a. The UV spectrum of the compound was almost identical with that of 2a except for the absorption intensities at 248 m $\mu$  due to a sulfoxide group. The IR spectrum of the compound was quite similar to that of 2a except that the peak at 1073 cm<sup>-1</sup> due to a sulfoxide group in the latter compound shifted to 1065 cm<sup>-1</sup> in the former. From the results obtained the compound is

supposed to be a lipoic acid-6-sulfoxide (8) or a stereoisomer, cis-(9) or trans-(10) isomer of 2a. In relation to latter consideration Johnson<sup>12</sup> demonstrated that the peroxide-oxidation of 4-substituted thian gave a mixture of cis- and trans-isomers (25 : 75) of the sulfoxides and that some differences in sulfoxide absorption of these compounds were observed in the IR spectrum. The compound showed almost the same microbiological activity as  $\alpha$ - and  $\beta$ -lipoic acid against Streptococcus faecalis 10C1 and Corynebacterium bovis. It also behaves as a pyruvate oxidation factor in the usual manometric technique using cell suspension of Streptococcus faecalis 10C1.



Oxidation of  $\alpha$ -lipoic acid (1a) with an excess of hydrogen peroxide in acetic acid resulted in the formation of 6,8-disulfonellipoic acid (5).

Its IR spectrum exhibited characteristic bands assignable to a sulfone group at 1310 ( $\nu_{as}$  SO<sub>2</sub>) and 1140 cm<sup>-1</sup> ( $\nu_s$  SO<sub>2</sub>). This compound exerted neither promoting effect nor inhibitory action on the growth of Streptococcus faecalis 10C1.

### 2.3. Experimental

#### 2.3.1. Photosensitized Oxygenation of Methyl $\alpha$ -Lipoate (1b).

A solution of methyl  $\alpha$ -lipoate (1b) (2.20 g, 10 mmoles) in methanol (100 ml) containing rose bengal (30 mg) was irradiated using a 100 W tungsten lamp with a Pyrex cooling jacket at room temperature. During irradiation oxygen was bubbled through the solution in a closed circulating system and the consumption of oxygen was determined manometrically. Oxygen consumption was ceased when oxygen (130 ml, 5.2 mmoles) had been taken up within 15 hr. After removal of the solvent, the residue was chromatographed on a silica gel column (40 g). Elution with chloroform (1000 ml) yielded methyl  $\beta$ -lipoate (2b) (2.1 g, 89%) as a viscose oil, which was identical with an authentic sample prepared (by IR and TLC).

$\nu_{\text{max}}^{\text{neat}}$  1730 and 1065  $\text{cm}^{-1}$ ; NMR( $\text{CDCl}_3$ )  $\tau$ 6.48 (singlet, 3H), 6.05 (multiplet, 1H), 6.50-7.90 (multiplet, 6H), 8.10-8.70 (multiplet, 6H). (Found: C, 44.41; H, 6.88; S, 26.52.  $\text{C}_9\text{H}_{16}\text{O}_3\text{S}_2$  requires: C, 44.01; H, 6.50; S, 26.01%).

In the absence of rose bengal oxygen was not consumed and the starting material (1b) was recovered quantitatively.

#### 2.3.2. Photosensitized Oxygenation of Methyl $\beta$ -Lipoate (2b)

A solution of methyl  $\beta$ -lipoate (2b) (1.0 g, 4.5 mmoles) in methanol (100 ml) containing rose bengal (20 mg) was photooxidized under the same conditions described above for 10 hr. The mixture exhibited no oxygen absorption and the starting material (2b) was recovered quantitatively.

#### 2.3.3. Oxidation of $\alpha$ -Lipoic Acid (1a) with t-Butylhydroperoxide.

To a solution of  $\alpha$ -lipoic acid (1a) (1.0 g, 4.5 mmoles) in 5 ml of chloroform was added t-

butylhydroperoxide (2.5 g, 22.5 mmoles). The mixture was allowed to stand for 1 day at 40° under continuous stirring, then mixed with 50 ml of water. After 24 hr. the chloroform layer was separated. The chloroform was evaporated in vacuo and the resulting brown oily residue was subjected to ascending paper chromatography using n-butanol saturated with 2.8% aqueous ammonia as a solvent. The oxidation products were detected by spraying with KCN-nitroprusside reagent. The major product was found to be  $\beta$ -lipoic acid (2a). Radiochromatographic technique was also employed by using  $^{35}\text{S}$ -labeled  $\alpha$ -lipoic acid as a starting material. Lipoic acid-activity or anti-lipoic acid-activity was examined by bioautography using Streptococcus faecalis 10C1. The zone corresponding to the unknown substance at Rf 0.90 was cut off and was extracted with methanol. After removal of the methanol, 7 (50 mg, 5%) was obtained as an oil. The compound showed a single spot on the paper chromatogram.

$\nu_{\text{max}}^{\text{CHCl}_3}$  1753, 1310, 1180, and 1140  $\text{cm}^{-1}$ .

(Found; C, 49.20, H, 7.56.  $\text{C}_{12}\text{H}_{22}\text{O}_4\text{S}_2$  requires: C, 48.98; H, 7.48%).

7 was hydrolysed with 1N H<sub>2</sub>SO<sub>4</sub> to give 8-sulfone-lipoic acid (4). Anti-lipoic acid activity of 4 was examined by bioassay method using Streptococcus faecalis 10C1 as a test organism<sup>13</sup>.

2.3.4. Oxidation of  $\alpha$ -Lipoic Acid(1a) with hydrogen Peroxide.

A. With 2 Molar Ratio of Hydrogen Peroxide.

To a solution of dl- $\alpha$ -lipoic acid (1a) (1.0 g, 4.5 mmoles) in the mixture of acetone (7 ml) and water (3 ml) 30% hydrogen peroxide (1 ml, 9 mmoles) was added. The mixture was stirred for 1 day at room temperature. The mixture was diluted with water and adjusted to pH 1.0 by the addition of 6N HCl. To the mixture chloroform was added, and the chloroform layer was separated. After removal of the solvent, the residual yellow oil was subjected to ascending paper chromatography using n-butanol saturated with 2.8% aqueous ammonia. Spots having the lipoic acid-activity in bioautography were observed at Rf 0, 0.2-0.4, 0.45 (remaining  $\alpha$ -lipoic acid (1a)), and 0.9. The zone corresponding to Rf 0.2-0.4

was extracted with methanol and rechromatographed on a broad filter paper using water-saturated n-butanol as a solvent. On the chromatogram lipoic acid-active substances were detected at Rf 0.4, 0.7, and 0.9. The zones at Rf 0.4 and 0.7 were extracted with methanol. After removal of the solvent,  $\beta$ -lipoic acid (2a) (70 mg) and an unidentified compound (30 mg) were obtained from the zone of Rf 0.4 and of Rf 0.7, respectively. The compound thus obtained showed a single spot on a paper chromatogram.  $\nu_{\text{max}}^{\text{CHCl}_3}$  1710 and 1065  $\text{cm}^{-1}$ . (Found: C, 43.43; H, 6.92.  $\text{C}_8\text{H}_{14}\text{O}_3\text{S}_2$  requires: C, 43.24; H, 6.31%).

The microbial activity of the compound was examined by bioassay method using Streptococcus faecalis 10C1 and Corynebacterium bovis as the test organisms. The pyruvate oxidation activity was also examined by the usual manometric technique using cell suspension of Streptococcus faecalis 10C1.

#### B. With an Excess of Hydrogen Peroxide

To a solution of  $\alpha$ -lipoic acid (1a) (0.2 g, 0.9 mmoles) in glacial acetic acid (10 ml) 30% hydrogen peroxide (1 ml, 9 mmoles) was added. The mixture was



kept at 40° for 1 day. After removal of acetic acid in vacuo, a white precipitate was obtained from the residue. Recrystallization from acetic acid gave 6,8-disulfonellipoic acid (5) as crystals, m.p. 108-110°.  $\nu_{\text{max}}^{\text{KBr}}$  1340 and 1140  $\text{cm}^{-1}$ . (Found: C, 35.60; H, 5.21.  $\text{C}_8\text{H}_{14}\text{O}_6\text{S}_2$  requires: C, 35.60; H, 5.19%).

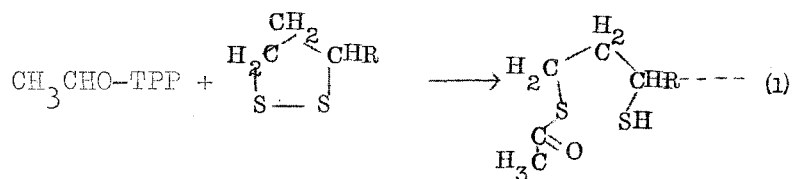
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### 3. Reaction of $\beta$ -Lipoic Acid with Acetic Anhydride as a Possible Model for the Enzymatic Acyl-Transfer Reaction

#### 3.1. Introduction

It is well known that  $\alpha$ -lipoic acid is an important cofactor concerning with the metabolism of pyruvic acid in biological systems<sup>1</sup>. In the pyruvate oxidation systems decarboxylation of the thiamine-pyruvate adduct yields an intermediate, active acetaldehyde ( $\text{CH}_3\text{CHO-TPP}$ ), which then reacts with  $\alpha$ -lipoic acid to form acetyl lipoic acid as shown in eq. 1<sup>1</sup>.



Although  $\beta$ -lipoic acid<sup>2-6</sup>(1) exhibits the same pyruvate oxidation activity as  $\alpha$ -lipoic acid, the role of  $\beta$ -lipoic acid (1) in the enzyme system catalized acyl-transfer reaction remains unknown. The well-known reactivity of the methylene carbon adjacent to a sulfoxide group would suggest that

the reactivity of 8-position of  $\beta$ -lipoic acid may be responsible for the enzymatic acyl-transfer reaction.

As a possible model of the acyl-transfer reaction the reaction of  $\beta$ -lipoic acid (1) with acetic anhydride was examined.

### 3.2. Results and Discussion

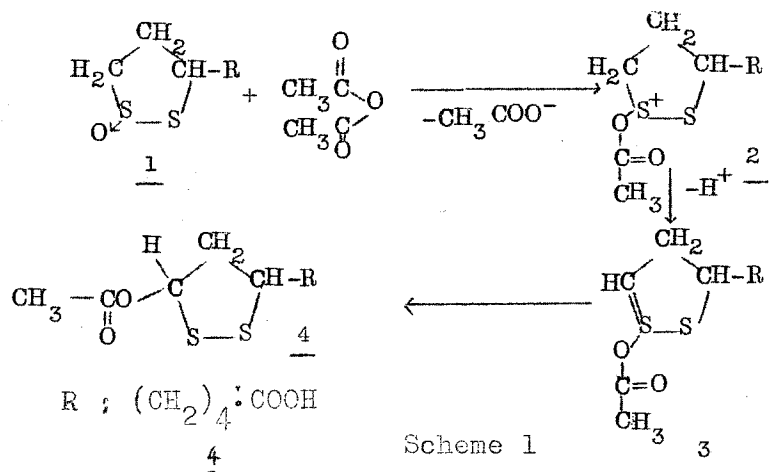
Reaction of  $\beta$ -lipoic acid (1) with acetic anhydride under mild conditions gave an unknown product (Rf 0.55),  $\alpha$ -lipoic acid (Rf 0.45) and the unchanged starting material (1) (Rf 0.30), which were detected on paper chromatography using solvent A. When  $^{35}\text{S}$ - $\beta$ -lipoic acid (1) was allowed to react with unlabeled acetic anhydride or when unlabeled  $\beta$ -lipoic acid (1) was reacted with  $^{14}\text{C}$ -acetic anhydride, the radioactivity was detected on the spot of the compound having Rf 0.55. The UV spectrum of the compound had no absorption maximum at 248  $\text{m}\mu$  due to a sulfoxide group but a weak peak at 330  $\text{m}\mu$  analogous to that of  $\alpha$ -lipoic acid (Fig. 1). The IR spectrum exhibited strong absorptions attributed to an acetoxy group at 1735 ( $\text{C}=\text{O}$ ) and 1300-1200  $\text{cm}^{-1}$ , and no absorption in

the sulfoxide region ( $1073\text{ cm}^{-1}$ ). On hydrolysis with alkali the product liberated acetic acid, which was detected by paper chromatography after conversion to the corresponding hydroxamate (Rf 0.48 in solvent C) and its ammonium salt (Rf 0.28 in solvent A). The results described above suggest that the compound is 8-acetoxylipoic acid (4), which is formed via the mechanism similar to that of the Pummerer reaction<sup>6,7</sup>.

In order to examine the possibility of the elimination of a proton attached to the carbon adjacent to the sulfoxide group of  $\beta$ -lipoic acid, deuterium exchange experiments were performed with  $\beta$ -lipoic acid methyl ester by NMR spectrometry. Methyl  $\beta$ -lipoate treated with  $\text{OD}^-$  in  $\text{D}_2\text{O}$  showed the identical behaviours with the untreated compound on paper chromatography. It also gave the similar infrared spectrum to that of the untreated compound, except that the weak bands assignable to C-D were observed at  $2,020$  and  $1,000\text{ cm}^{-1}$ . In its NMR spectrum among the multiplet from  $\tau 6.7$  to  $7.3$  (relative area 3.0)

attributed to the activated methylene protons as well as the methine proton owing to the adjacent sulfur atom, a peak at  $\tau$  6.7 (relative area 0.8) disappeared completely after the deuteration. The result would indicate the average value of the exchange of two methylene protons at C-8 position or a predominant exchange of one of them.

The formation of 8-acetoxylipoic acid (4) from  $\beta$ -lipoic acid (1) and acetic anhydride is well rationalized by the similar pathway as that of Pummerer reaction <sup>6,7</sup>(Scheme 1). The attack of acetyl cation on 1 gives 2, which liberates a proton to give S-acetoxy derivative (3). 3 then rearranges to form 8-acetoxylipoic acid (4). Deuterium exchange experiments des-



cribed above would support the above mechanism.

The chromatographic behaviors of 8-acetoxy-lipoic acid (4) compared with those of  $\alpha$ -lipoic acid and  $\beta$ -lipoic acid (1) was shown in Table 1. 8-Acetoxy-lipoic acid (4) exhibited almost the same microbiological activity for Streptococcus faecalis 10C1 as  $\alpha$ - and  $\beta$ -lipoic acid (Fig. 2). Associated with the well-known competitive inhibitory effect of 8-methyl-lipoic acid as observed by Stokstad<sup>10</sup>, the results obtained in this study would suggest the possibility that the reactivity at C-8 position of  $\beta$ -lipoic acid (1) may play an

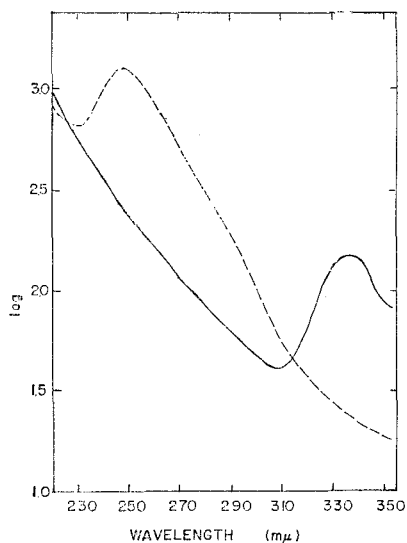


Fig. 1: Ultraviolet Spectrum of  $\alpha$ -Lipoic Acid(1) and 8-Acetoxy-lipoic Acid(4)(in methanol)  
-----, (4)      -----, (1).

interesting role in biological systems.

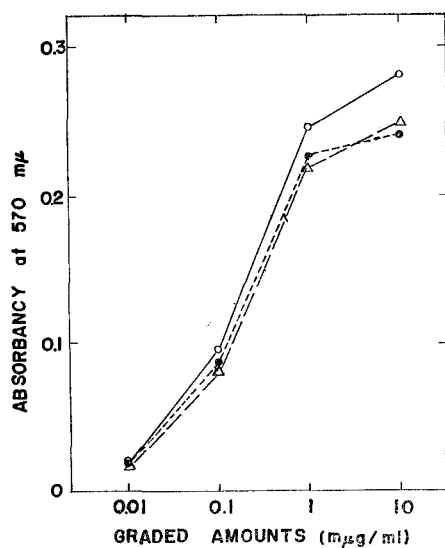


Fig. 2: Microbiological Activity of 8-Acetoxy-lipoic Acid (4).  
Test organism, Streptococcus faecalis 10C1.  
O, 4; ⊙, α-lipoic acid; Δ, β-lipoic acid.  
Incubation was carried out for 16 hr at 37°.

Table 1

Chromatographic Behaviors of 8-Acetoxy-lipoic Acid (4)

Chromatographic system	Solvent	Rf value		
		α-Lipoic acid	β-Lipoic acid	8-Acetoxy-lipoic Acid(4)
Paper	A	0.45	0.30	0.55
	B	0.92	0.60	0.90
	C	0.95	0.85	0.95
Silica gel G	D	0.93	0.90	0.75



Solvent A, 2.8% aqueous ammonia-saturated n-butanol; Solvent B, water-saturated n-butanol; Solvent C, n-butanol-acetic acid-water (4:1:5). Ascending paper chromatography was carried out for 15 to 17 hr at 25°. Solvent D for thin layer chromatography is petroleum ether-benzene-acetone (5:5:1). Spots were detected with KCN-nitroprusside reagent as well as a bioautographic technique using Streptococcus faecalis 10 C1 as a test organism.

### 3.3. Experimental

#### 3.3.1. Materials

$\alpha$ -Lipoic acid and  $^{35}\text{S}$ -labelled  $\alpha$ -lipoic acid (specific activity, 420  $\mu\text{c}/\text{mmole}$ ) were kindly supplied from Fujisawa Pharmaceutical Industries, Inc., Osaka. 1- $^{14}\text{C}$ -Labeled acetic anhydride (specific activity, 5  $\text{mc}/\text{mmole}$ ) was commercially available.

#### 3.3.2. Paper chromatography and Thin Layer Chromatography

Ascending paper chromatography was carried out for 15 to 17 hours at 25°, and the spots were

detected by spraying with KCN-nitroprusside reagent as well as a bioautographic technique using Streptococcus faecalis 10C1 as a test organism. The following solvents were used: Solvent A, 2.8% aqueous ammonia-saturated n-butanol; Solvent B, water-saturated n-butanol; Solvent C, n-butanol-acetic acid-water (4:1:5).

Thin layer chromatography was performed on a silica gel G using petroleum ether-benzene-acetone (5:5:1) as a developing solvent system for 1 to 2 hours at 25°. The zones of the lipoic acid-related substance were detected in the same way as on paper chromatography. When labelled  $\alpha$ -lipoic acid or labelled acetic anhydride was used, the spots on the paper were detected using the instrument of Nuclear-Chicago, No. 1620.

### 3.3.3. Growth Rate Studies

Lipoic acid activity was studied according to the procedure of Stokstad<sup>9</sup> using Streptococcus faecalis 10 C1 and Corynebacterium bovis. In this case, the samples were added aseptically to the medium without heat sterilization in order to avoid possible decomposition as far as possible. The growth of the micro-

organism was measured turbidmetrically and expressed by the absorbance at 570 m $\mu$ .

#### 3.3.4. Reaction of $\beta$ -Lipoic Acid with Acetic Anhydride

To a solution of  $\beta$ -lipoic acid (1)(300 mg, 1.4 mmoles) in chloroform (2 ml) and ether (4 ml) acetic anhydride (450 mg, 4.4 mmoles) was added. The mixture was kept for 2 days at 45° in nitrogen atmosphere. After the reaction was furnished, ether, chloroform, resulting acetic acid and an excess of acetic anhydride were removed below 40° in vacuo. The brown oily residue was subjected to ascending paper chromatography with a broad filter paper using solvent A. The zone of an unknown reaction product at Rf 0.55, detected in addition to  $\alpha$ -lipoic acid and  $\beta$ -lipoic acid (1), was extracted with chloroform and further purified by thin layer chromatography (silica gel G) using petroleum ether-benzene-acetone (5:5:1) as a solvent. The reaction product was obtained as a light-yellow oily substance (4)(20 mg, 6%).  $\lambda_{\text{max}}^{\text{MeOH}}$  255 m $\mu$ (log 3.18);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3 1735, 1365, 1300-1200, and 1040 cm<sup>-1</sup>. (Found: C, 45.22; H,

6.58.  $C_{10}H_{16}O_4S_2$  requires: C, 45.45; H, 6.06%).

This compound showed almost the same microbiological activity for Streptococcus faecalis 10C1 and Corynebacterium bovis as  $\alpha$ - and  $\beta$ -lipoic acid (1) (Fig. 2).

### 3.3.5. Deuterium Exchange Studies

To a suspension of methyl  $\beta$ -lipoate (200 mg, 0.9 mmole) in  $D_2O$  (5 ml) metallic sodium (13 mg, 0.57 mmole) was added. The mixture was allowed to stand for 24 hr at room temperature with vigorous stirring. The resulting product was extracted with carbon tetrachloride. The compound thus obtained showed the same behaviours as methyl  $\beta$ -lipoate on paper chromatography and thin layer chromatography.  $\nu_{\max}^{CHCl_3}$  2020 and 1000  $cm^{-1}$  (C-D).

NMR( $CCl_3$ );  $\tau$  6.50 (singlet, 3H,  $-OM_e$ ), 8.00-8.70 (multiplet, 6H), 7.60-7.90 (multiplet, 4H) and 6.70-7.30 (multiplet, 2H).

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## CHAPTER IV

### PHOTOSENSITIZED OXYGENATION OF 2,4,5-TRIPHENYL THIAZOLE

#### 1. Introduction

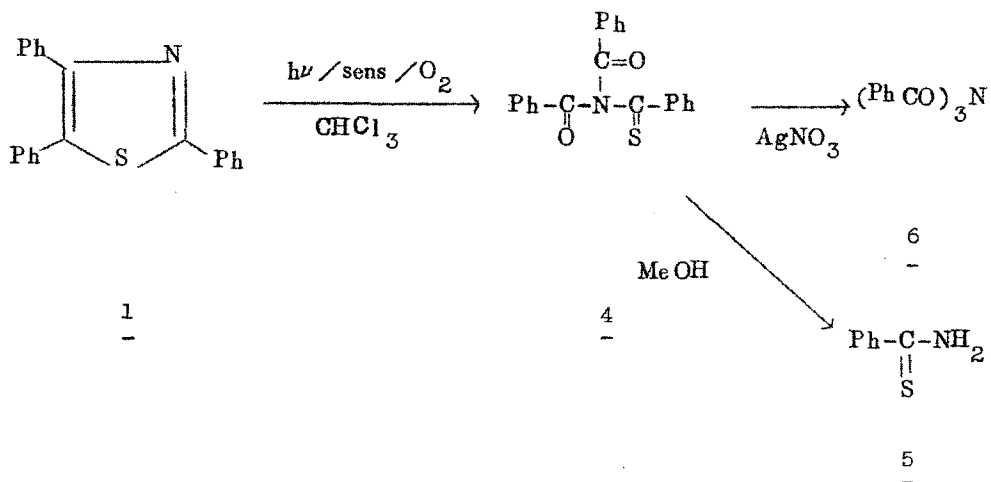
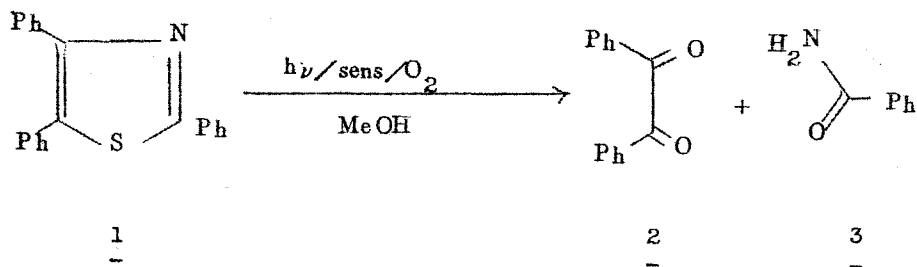
Recently much attention has drawn to the photosensitized oxygenation of five-membered heterocyclic bases in relation to the photodynamic action of the biological systems<sup>1</sup>. Although the photosensitized oxygenation of imidazoles<sup>2</sup>, Pyrroles<sup>3</sup>, oxazoles<sup>4</sup>, indoles<sup>5</sup> and purines<sup>6</sup> has been studied extensively, the photooxidation of thiazole, which is an important part of thiamine, has never been investigated. In this chapter, the photosensitized oxygenation of 2,4,5-triphenylthiazole are described. The results provide further information regarding the structure of the peroxide intermediate and the manner of its decomposition in the photosensitized oxygenation of five-membered heterocyclic compounds.

## 2. Results and Discussion

When a methanol solution of 2,4,5-triphenylthiazole(1) was submitted to photosensitized oxygenation in the presence of rose bengal, 0.45 mole of oxygen was consumed for 24 hr and benzil(2) and benzamide(3) were obtained in 11 and 18 % yield, respectively.

On the other hand, when photosensitized oxygenation was carried out in chloroform using methylene blue as a sensitizer, 1 afforded a different product. Thus, 1 gave, after consumption of 0.9 mole of oxygen, a red colored compound  $C_{21}H_{15}NO_2S$ , an oxygen adduct of 1, in 30 % yield. The IR spectrum shows a broad carbonyl absorption band at  $1700\text{ cm}^{-1}$ . In its UV spectrum a weak absorption maximum at  $500\text{ m}\mu$  was observed. Treatment of the adduct with boiling methanol gave thiobenzamide(5) in 50 % yield. The adduct yielded tribenzamide(6) in aqueous silver nitrate solution in 10 % yield. These reactions and spectral data described above are known to be generally found in the case of N-acylthiobenzamides<sup>7</sup>. The above results led us to assign structure 4 for

the adduct.

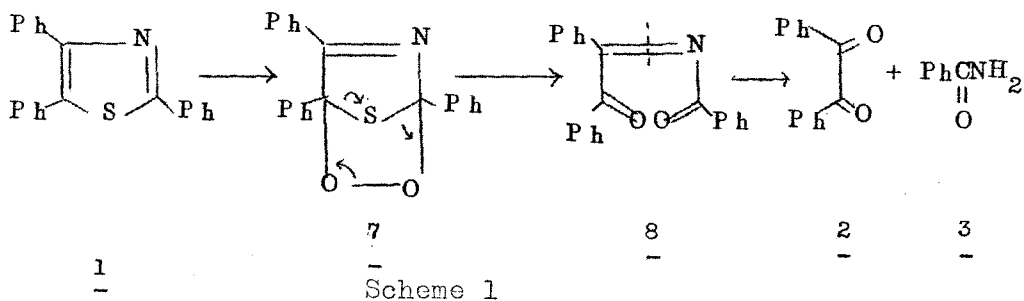


In the absence of the sensitizer, 1 did not consume oxygen and the starting material(1) remained unchanged. This indicates that the presence of a



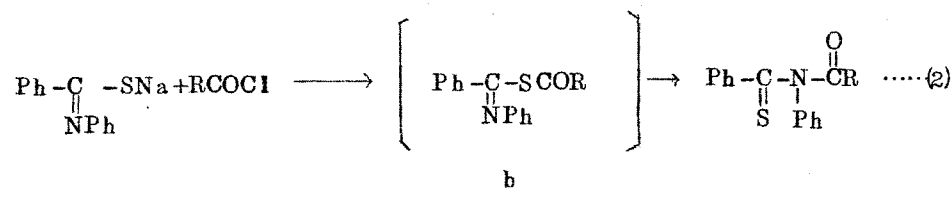
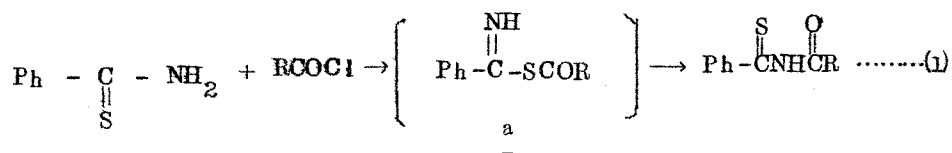
sensitizer is a requisite condition for the present photooxygenation.

The formation of benzil(2) and benzamide(3) in the photosensitized oxygenation of 1 in methanol is well rationalized by a pathway involving a cyclic peroxide 7(Scheme 1). Such a cyclic peroxide is commonly recognized as an intermediate in the photosensitized oxygenation of five-membered heterocycles, i.e., imidazoles<sup>2</sup>, pyrroles,<sup>3</sup> oxazoles<sup>4</sup>, isoindoles<sup>8</sup> and isobenzothiophene.<sup>9</sup> The initially formed peroxide 7 could undergoes rearrangement to form a shiff base 8, which is hydrolysed to give 2 and 3. The loss of the sulfur atom of 7 has analogy in the photosensitized oxygenation of 1,3-diphenylisobenzothiophene leading to the o-debenzoylbenzene.<sup>9</sup>



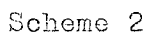
In inert solvents such as chloroform, the photosensitized oxygenation of 1 takes a different

course. Such a solvent effect on the photooxidation is also observed in the cases of purines<sup>6</sup> and oxazoles<sup>10</sup>. Two mechanisms can be considered to account for the formation of 4 from 1. In this case a zwitterionic peroxide 9a or 9b<sup>2,6,11</sup> is proposed for the first step intermediate. As shown in Scheme 2, 9 could rearrange to a four-membered cyclic peroxide 10 (path a), which undergoes ring cleavage to give N-benzoyl-S-benzoyl - isothiobenzamide(11). Such a photooxidative cleavage of c=c double bond has analogy in the photooxidation of 1,2,3,4-tetraphenylpyrrole leading to  $\alpha$ -N-benzoyl-amino- $\alpha'$ -benzoylstilbene.<sup>12</sup> The isothiobenzamide (11) would then undergo further intramolecular rearrangement to give 4. Such types of rearrangement has been proposed in the reaction of acyl chloride with thio-benzamide (eq. 1)<sup>7</sup> and N-phenylthiobenzamide (eq. 2)<sup>13</sup>, respectively. N-Benzoylisothiobenzamide(a) in the former and N-phenyl-S-benzoylisothiobenzamide (b) in the latter case have been considered to be an intermediate.



An alternative pathway (path b) including a cyclic peroxide 7, which has analogy in the photo-sensitized oxygenation of oxazole<sup>4</sup>, may also account for the formation of 4. The peroxide 7 could rearrange to give 12, which undergoes an intramolecular rearrangement, analogous to N-benzoyl-isouimide, to give 4<sup>4</sup>. The formation of the endo-peroxide 7 may occur either directly from 1 or stepwise via the zwitterionic peroxide 9a.

Although from the available results it cannot be distinguished between the above two modes of formation of 4, path a seems to be more favorable considering from the reaction of eq. 2.



### 3. Experimental

#### 3.1. Photosensitized Oxygenation of 2,4,5-triphenylthiazole (1)

##### A. In Methanol

A solution of 2,4,5-triphenylthiazole<sup>14</sup> (1) (2.8 g, 9 mmoles) in methanol (300 ml) containing rose bengal (50 mg) was irradiated at room temperature by a 100 W high-pressure mercury lamp through a Pyrex cooling jacket. During irradiation oxygen was bubbled by a circulating pump through a sintered-glass joint which was attached at the bottom of the reaction vessel. Oxygen consumption was manometrically followed. Oxygen absorption was ceased after oxygen (105 ml, 4.2 mmoles) had been taken up for 24 hr. After removal of the solvent in vacuo, the residue was chromatographed on a neutral alumina column (50 g). Elution with chloroform (400 ml) gave unchanged starting material (1) (0.95 g, 34% recovery). Further elution with chloroform (Ca. 600 ml) yielded benzil (2) (135 mg, 11% based on the reacted starting material). Recrystallization from ethanol gave yellow prisms,

m.p. 93-94°, which were identical with an authentic sample by a comparison of their IR spectra. Chloroform (1000 ml) eluted benzamide (3) (95 mg, 18% based on reacted 1). Recrystallization from ethanol gave crystals, m.p. 128°.

#### B. In Chloroform

A solution of 2,4,5-triphenylthiazole (1) (2.4 g, 7.7 mmoles) in dry chloroform (200 ml) containing methylene blue (50 mg) was irradiated by a 100 W high-pressure mercury lamp for 10 hr described above, until oxygen (170 ml, 6.8 mmoles) was consumed. The solvent was removed in vacuo, and the residue was dissolved in ether (100 ml). The ethereal solution was treated with Norit. After removal of the solvent, the residue was crystallized from ether to give N,N-dibenzoylthio-benzamide (4) (0.78 g, 30%). Recrystallization from ether (5 ml) gave red colored crystals, m.p. 102°-103°.

$\lambda_{\text{max}}^{\text{cyclohexane}}$  224 m $\mu$  ( $\epsilon$  29000), 233 ( $\epsilon$  25000), and 500 ( $\epsilon$  150);  $\nu_{\text{max}}^{\text{nujol}}$  1700, 1600, and 1300-1200 cm<sup>-1</sup>.

(Found: C, 73.43; H, 4.47; N, 4.08; S, 9.24. C<sub>21</sub>H<sub>15</sub>NO<sub>2</sub>S requires: C, 73.04; H, 4.63; N, 4.06; S, 9.28%).

### 3.2. Reactions of N,N-Dibenzoylthiobenzamide (4)

#### A. With Methanol

A solution of 4 (100 mg) in methanol (40 ml) was boiled for 10 min. A thin layer chromatographic analysis (silica gel, chloroform-ethanol (20:1)) of the mixture showed that it consisted of at least five compounds among which thiobenzamide (5), (20 mg, 50 %) was isolated by preparative thin layer chromatography.

#### B. With Silver Nitrate

To a suspension of 4 (100 mg) in the mixture of methanol (10 ml) and water (10 ml) silver nitrate (400 mg) was added, according to the procedure of Horstmen et al<sup>7</sup>. The solution was acidified, diluted with water (50 ml), and then extracted with ether (100 ml). After removal of ether, ethanol was added to the residue. The ethanol-insoluble solid was collected by filtration and was crystallized from boiling ethanol (ca. 80 ml) to give tribenzamide (6) (10 mg, 10 %), m.p. 198-200° (lit<sup>15</sup>., m.p. 201-202°), which was identical with the authentic sample prepared according to the method of Curtius<sup>15</sup> (by IR).

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## SUMMARY

The author's studies collected in this volume are concerned with the oxidation, particularly photosensitized oxidation, of some nitrogen- and sulfur-containing heterocycles with biochemical significance. Some new findings which have important biological implications have been gained by the present investigations.

### Chapter I. Photosensitized Oxygenation of Purine Derivatives

In connection with the photodynamic inactivation of deoxyribonucleic acids, the photosensitized oxygenation of purine derivatives has recently drawn much attention. In order to contribute to the elucidation of the photodynamic degradation of deoxyribonucleic acids, a systematic investigation on the photosensitized oxygenation of purine derivatives has been carried out.

The photosensitized oxygenation of some N-unsubstituted hydroxypurines in aqueous alkaline solution in the presence of rose bengal as a sensitizer was investigated. Under these conditions, xanthine gave allantoin and triuret, uric acid gave triuret, sodium oxonate and allantoxaidin, and

8-methylxanthine resulted in the formation of a complex mixture from which acetamide and sodium oxonate were isolated. The mechanisms for these reactions, including peroxide intermediates, are discussed. These products are possibly formed via a peroxide intermediate. Thus, the formation of allantoin from xanthine can be well rationalized by considering a 5, 8-endo-peroxide intermediate (2) (section 2).

Photosensitized oxygenation of 9-phenylxanthine and 1, 3-dimethyl-9-phenylxanthine in methanol in the presence of rose bengal gave the corresponding 4, 5-dimethoxyuric acid derivatives. The results indicate the intermediary formation of a 4, 8-endo-peroxide (4) in the reaction. Under similar conditions, 1, 3-dimethyl-9-phenyluric acid and 9-phenyluric acid also yielded the corresponding 4, 5-dimethoxyuric acid derivatives. Possible mechanisms involving peroxide intermediates, a 4, 8-endo-peroxide (4) and a 4-hydroperoxide (6), are discussed (section 3).

Photosensitized oxygenation of 1, 3, 7, 9-tetramethyluric acid in methanol yielded 4, 5-dimethoxy-1, 3, 7, 9-tetramethyluric acid. On the other hand, photosensitized oxygenation of fully N-alkylated uric acids in chloroform

in the presence of methylene blue resulted in the formation of different types of products. Thus, 1, 3, 7, 9-tetramethyluric acid gave 1, 3, 7-trimethylcaffolide and 1-ethyl-3, 7, 9-trimethyluric acid gave 1, 3-dimethyl-7-ethylcaffolide and a nine-membered ring compound. For the first step intermediate in these reactions a zwitterionic peroxide 8 is proposed. In order to elucidate whether singlet oxygen is involved in the photooxidation reaction, reaction of these fully N-alkylated uric acids with singlet oxygen, which was generated by non-photochemical means, was also investigated. From the results obtained it is concluded that the zwitterionic peroxide may be formed at least in part by the attack of singlet oxygen to the tetraalkyluric acids in the photosensitized oxygenation (section 4).

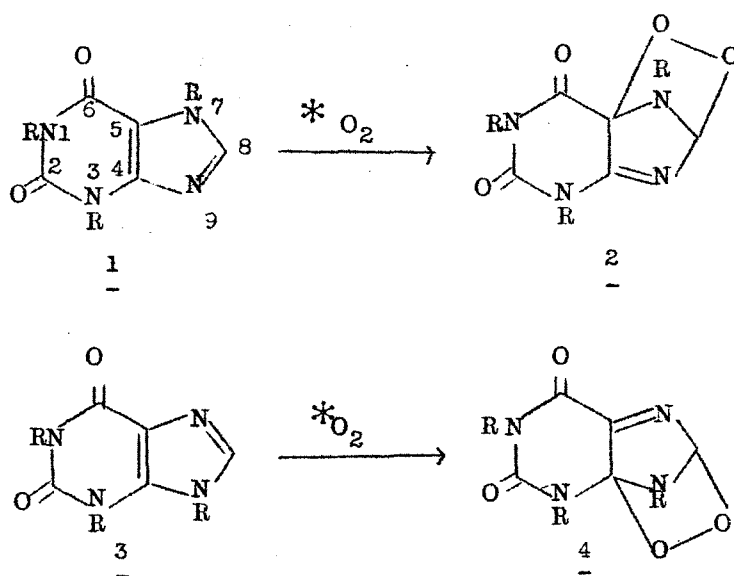
Photosensitized oxygenation of 8-methoxycaffeine in methanol containing rose bengal yielded carbon dioxide and 1-methyl-2, 2-dimethoxy-4-methylamino-3-imidazolin-5-one in good yield. Similarly, 8-methoxycaffeine in ethanol gave carbon dioxide, ethyl N-methylcarbamate, and 1-methyl-2-ethoxy-methoxy-4-methylamino-3-imidazolin-5-one. Photooxygenation of other N-alkylated 8-alkoxyxanthines, which gave the

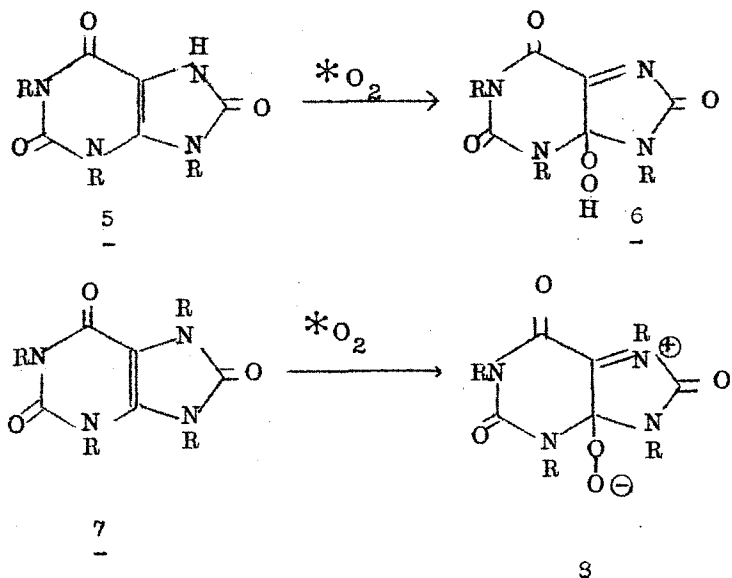
corresponding imidazolinones, was carried out in connection with the mechanisms of these reactions. The detailed reaction sequences involving a 5, 8-endo-peroxide (2) for the formation of the imidazolinone from 8-alkoxycaffeine have been clarified from the results obtained (section 5).

Photosensitized oxygenation of 9-phenylguanine, a simple analogue of guanosine, in an aqueous alkaline solution afforded carbon dioxide, guanidine, phenylurea, and a trace of phenylparabanic acid. The results obtained in the photosensitized oxygenation of various purine derivative (section 2-5) led us to propose possible mechanisms for the photodynamic degradation of guanine and guanosine. In these reactions a 5, 8-endo-peroxide as 2 in the former and a 4, 8-endo-peroxide as 4 in the latter are an intermediate (section 6).

From all the above results (section 2-6), following conclusions may be given to the photosensitized oxygenation of purine derivatives. (1) The attack of singlet oxygen to the ground state molecule of the purines may result in the initial formation of a peroxide intermediate. (2) The nature of the peroxide is depending upon the structural feature of the imidazole moiety of the purines. Thus,

four types of peroxides, i.e., a 5, 8-endo-peroxide (2), a 4, 8-endo-peroxide (4), a 4-hydroperoxide (6), and a zwitterionic peroxide (8), may be formed. (3) The decomposition modes of the peroxide is depending upon its surroundings to give various types of products.





## Chapter II. Oxidation of Quinoxaline-2, 3-diols as a Possible Model for the Biological Decomposition of Riboflavin.

In connection with the biological decomposition of riboflavin, the oxidation of three quinoxaline-2, 3-diol derivatives, i.e., 1-ribityl-2, 3-diketo-6, 7-dimethyl-1, 2, 3, 4-tetrahydroquinoxaline (1), 6, 7-dimethylquinoxaline-2, 3-diol (2), and quinoxaline-2, 3-diol (3) was examined by chemical means. The former two compounds are known to be catabolic intermediates of riboflavin.

On oxidation with alkaline ferricyanide, quinoxaline-2, 3-diol (3) gave cis,trans-muconic acid and a dimer, 6, 7-dimethylquinoxaline-2, 3-diol (2) gave 3, 4-dimethyl-

4-carboxymethyl- $\Delta$ '-butenolide (4) and 6-carboxy-7-methyl-quinoxaline-2, 3-diol (5), and 1-ribityl-2, 3-diketo- 6,7-dimethyl, 2, 3, 4-tetrahydroquinoxaline (1) gave 2, 4, and 5.

The mechanism for the formation of 4 from 2 is discussed in connection with the enzymatic degradation of riboflavin. In this mechanism a common intermediate, 4, 5-dimethylbenzoquinone, in both the enzymatic and chemical degradation of 2, is proposed (section 1).

Whereas 3 was resistant to photosensitized oxygenation, both 1 and 2 were destructively degraded under similar conditions. However, 2 was found to be an intermediate in the photooxygenation of 1. The mechanism for the formation of 2 from 1 by photosensitized oxygenation is discussed in comparison with that of the enzyme catalyzed reaction of 1 (section 2).

### Chapter III. Oxidation of Lipoic Acid and its Model Reaction for the Enzymatic Acyl-Transfer Reaction

In order to contribute to the elucidation of the structure of the metabolites of lipoic acid, the oxidation of lipoic acid was investigated. The photosensitized oxygenation of  $\alpha$ -lipoic acid in methanol gave  $\beta$ -lipoic



acid. Mechanism of the reaction is briefly discussed. On oxidation with t-butylhydroperoxide, lipoic acid afforded 8-sulfonelipoic acid, which showed an inhibitory effect on Streptococcus faecalis 10C1 with a 50% inhibition index of about 100. Peracetic acid oxidation of lipoic acid gave 6, 8-disulfonelipoic acid, which exerted neither promoting nor inhibiting effect on the growth of Streptococcus faecalis 10C1 (section 1).

Moreover, reaction of  $\beta$ -lipoic acid with acetic anhydride under mild conditions gave 8-acetoxylipoic acid, which showed almost the same microbiological activity as  $\alpha$ - and  $\beta$ -lipoic acid. These reactions are discussed in connection with the enzymatic acyl-transfer reaction, in which lipoic acid plays an important role (section 2).

#### Chapter IV. Photosensitized Oxygenation of 2, 4, 5-triphenylthiazole

In connection with the photodynamic action of the biological systems, photosensitized oxygenation of thiazoles, which is an important part of thiamine, was investigated. Photooxidation of 2, 4, 5-triphenylthiazole in methanol in the presence of rose bengal resulted in

the formation of benzil and benzamide. On the other hand, photooxidation of 2, 4, 5-triphenylthiazole in chloroform containing methylene blue as a sensitizer gave N, N-dibenzoylthiobenzamide in good yield. Possible mechanisms for these reactions, including an endo-peroxide or a zwitterionic peroxide, are discussed.

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